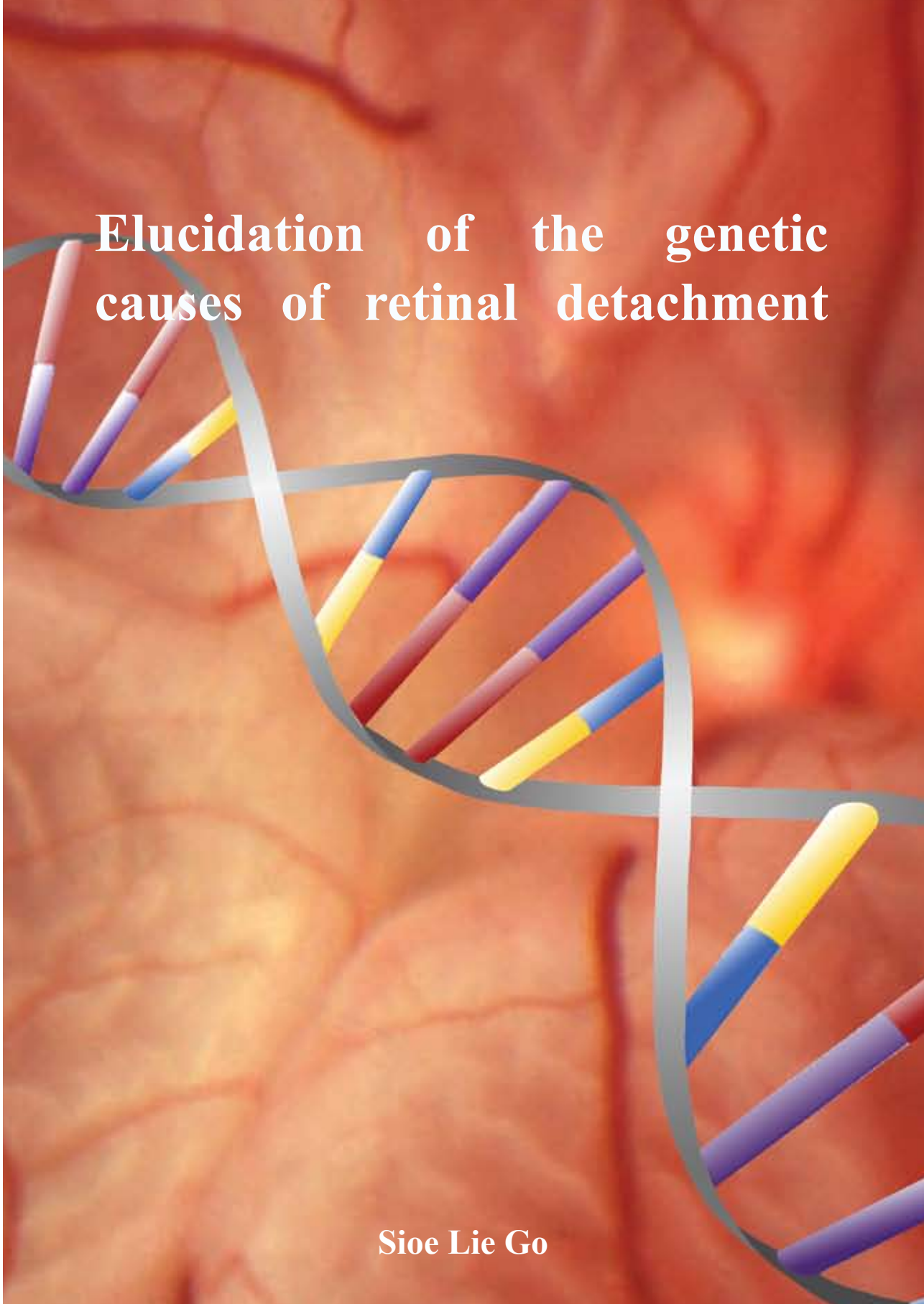
Elucidation of the genetic causes of retinal detachment



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Elucidation of the genetic causes of retinal detachment

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op het gebied van de Medische Wetenschappen

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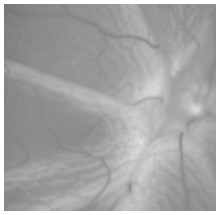

Voor mijn ouders
Voor Milan

Contents

List of abbreviations

ad	autosomal dominant
ADS	auricula dexter et sinister
cDNA	complementary DNA
COL2A1	collagen type II, alpha-1 chain gene
COL5A2	collagen type V, alpha-2 chain gene
COL9A1	collagen type IX, alpha-1 chain gene
COL9A2	collagen type IX, alpha-2 chain gene
COL9A3	collagen type IX, alpha-3 chain gene
COL11A1	collagen type XI, alpha-1 chain gene
COL11A2	collagen type XI, alpha-2 chain gene
COL18A1	collagen type XVIII, alpha-1 chain gene
D	diopter(s)
DNA	deoxyribonucleic acid
ERG	electroretinogram
EVR1	exudative vitreoretinopathy locus number 1
EVR2	exudative vitreoretinopathy locus number 2
EVR3	exudative vitreoretinopathy locus number 3
FBN1	fibrillin-1 gene
FZD4	frizzled-4 gene
IL1B	interleukin 1, beta
LRP5	low-density-lipoprotein receptor-related protein 5 gene
mRNA	messenger RNA
MYP1	myopia locus 1
MYP2	myopia locus 2
MYP3	myopia locus 3
MYP4	myopia locus 4
MYP5	myopia locus 5
NCRNA	Nonsyndromic congenital retinal nonattachment (locus)
Nd	neodymium
ND	Norrie disease gene
OD	oculus dexter
OS	oculus sinister
PCR	polymerase chain reaction
PVD	posterior vitreous detachment
PVR	proliferative vitreoretinopathy
RNA	ribonucleic acid
RRD	rhegmatogenous retinal detachment
RPE	retinal pigment epithelium
RS1	retinoschisis 1 gene

RT-PCR	reverse transcription-PCR
SP1	transcription factor specificity protein 1 gene
SP3	transcription factor specificity protein 3
tel	telomere
WGN1	Wagner disease locus 1
YAG	yttrium aluminium garnate

Chapter

1

Introduction

1.1 Anatomy of the eye

Visual pathway
Light from an object enters the eye through the strongly light-refracting cornea (**Figure 1.1**) and passes the anterior chamber and the pupil, the small opening in the iris.

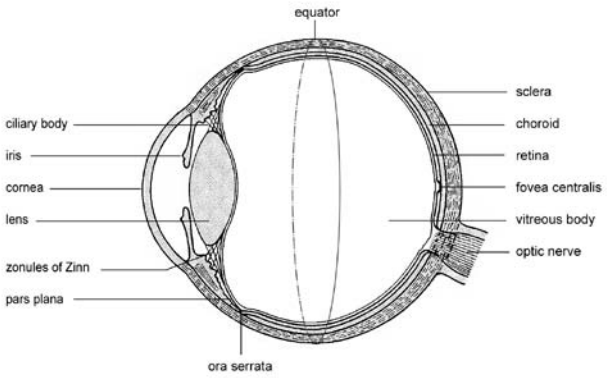

Figure 1.1 Anatomy of the eye

The pathway of the beams then continues through the lens, which ideally refracts the light in such a manner, that it is focussed on the photoreceptor cells in the retina after its passage of the vitreous body. The photoreceptor cells then convert the light into a neuronal signal that is transmitted to the visual brain cortex in the occipital lobes. The pathway in the retina leads from the photoreceptor cells, through the bipolar cells, to the ganglion cells. The axons of these cells form the optic nerve and -via the optic chiasm and the optic tract- extend to the lateral geniculate body. Finally, the optic radiation passes the signal on to the occipital cortex.
Horizontal communication between the parallel pathways in the retina is possible via the horizontal cells and amacrine cells.

Anatomy
The eye itself consists of three layers: the outer shell formed by the sclera, with at the frontside the transparent cornea, secondly the uveal layer consisting of the iris, ciliary body and the large choroid –the latter closely attached to the sclera, and containing the outer retina-nourishing choriocapillaris-, and finally the retinal layer. This layer is directly attached to the choroid and anteriorly ends in the ora serrata at the beginning of pars plana of the ciliary body. The central retinal vessels in this layer, originating from the central retinal artery that branches from the ophthalmic artery, nourish the inner part of the retina. In about 15% of all individuals, however, part of the posterior

pole of both eyes is supplied by a cilioretinal artery originating from the choroidal vasculature through the temporal part of the optic nervehead. Unilateral cilioretinal (co-)vascularisation of the posterior pole is found in an additional 34.9%.[1]

The inner eye structures divide the interior of the eye in three compartments, as visible in **Figure 1.2**. The anterior chamber is divided from the posterior chamber by the iris plane, which in its turn is divided from the vitreous cavity by the lens and the plane formed by the threadlike zonules of Zinn, that surround the periphery of the lens and keep it in its place. The other ends of the zonules are attached to the pars plicata of the ciliary body.

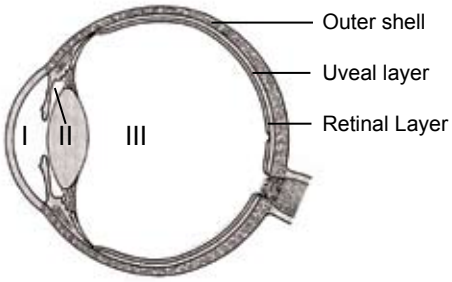

Figure 1.2 The three layers dividing the eye in three compartments

The anterior and posterior chambers are filled with the aqueous fluid, which is produced by the ciliary body, and which nourishes the lens and cornea. The balance between the production of the aqueous and the drainage via the trabecular meshwork in the sharp angle formed by the cornea and the iris in the anterior chamber results in a certain ocular pressure.

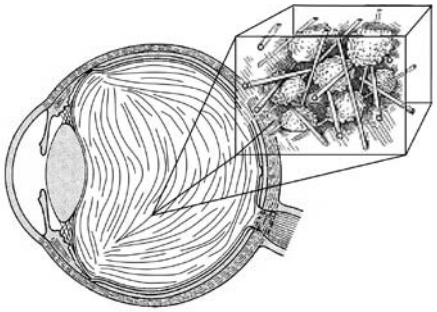

Figure 1.3 Soluble proteins and hyaluronic acid in the collagen fibre meshwork in the vitreous body

The vitreous cavity is filled with a transparent gel-like substance: the vitreous body, which consists of a large quantity of water (about 99%) with a solution of salts, soluble proteins and hyaluronic acid contained in a meshwork of collagen fibres, mostly of the type II collagen. (See **Figure 1.3**) In young healthy eyes, this substance fills the complete vitreous cavity and is in contact with its surrounding structures: the retina, the ciliary body, the lens zonulae and the posterior surface of the lens.

The vitreous is most strongly attached to the retina at the vitreous base: a 2-6 mm wide circumferential zone of concentrated collagen fibrils covering the ora serrata, which anteriorly extends to the adjacent epithelium of the pars plana and posteriorly extends to the peripheral retina. (See **Figure 1.4**) Along the peripheral margin of the optic nervehead, a second relatively strong attachment of the vitreous to the retina is formed by vitreous fibrils that intermingle with the thickened basement membrane of the retina, but it is less firm than at the vitreous base.

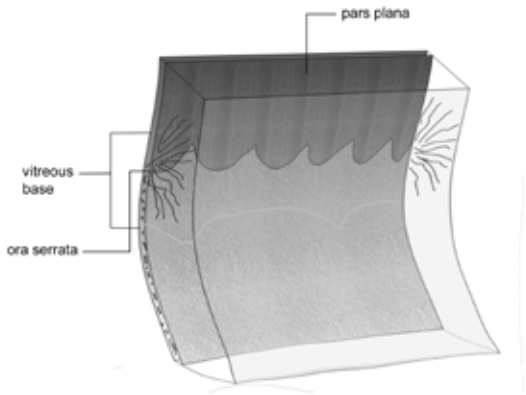

Figure 1.4 The vitreous base, covering the ora serrata

Histology of the retina
Histologically, the retina consists of ten layers (**Figure 1.5**). The retinal layer directly attached to the choroid is the retinal pigment epithelium (RPE). Both this layer and the tight junctions of the vascular endothelium of the capillaries form a strong barrier between the bloodcells and the photoreceptor cells. They are part of the blood-brain barrier.
The remaining nine layers form the sensory retina. Numbered from the outside to the inside these nine layers are: 1. the photoreceptor layer, containing the outer and inner segments of the rods and cones; 2. the external limiting membrane, composed of the closely apposed processes of Müller cells from the fifth layer; 3. the outer nuclear layer, containing the cell bodies of the photoreceptor cells; 4. the outer plexiform layer, the zone of synapses between the interneurons (horizontal, bipolar

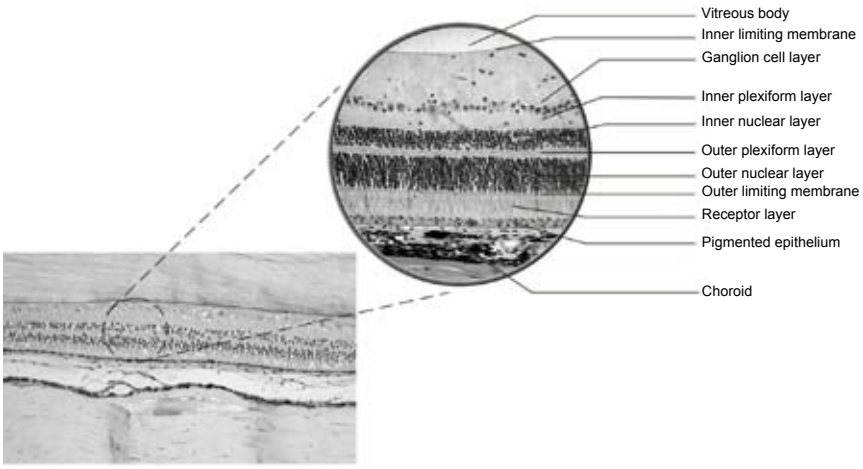

Figure 1.5 The ten layers of the retina and the adjacent choroid

and amacrine cells) and the photoreceptors; 5. the inner nuclear layer, composed of the cell bodies of the interneurons and Müller cells; 6. the inner plexiform layer, the zone of synapses between the interneurons and the ganglion cells; 7. the ganglion cell layer, containing the cell bodies of the ganglion cells; 8. the nerve fiber layer, composed of the unmyelinated axons of the ganglion cells that course towards the optic disc; and 9. the internal limiting membrane, like the external limiting membrane formed by the apposed processes of the same Müller cells. This pattern of layers is found throughout the retina, except in the fovea.

Certain parts of the posterior retina are described as the macula, the fovea and the foveola (**Figure 1.6**). The anatomic macula is the posterior part of the retina containing the yellow xanthophyll pigment and two or more layers of nuclei in the ganglion cell layer. The area, vertically centered between the temporal vascular arcades at about 4.0 mm temporal and 0.8 mm inferior to the center of the optic disc, is usually 5-6 mm in diameter and is responsible for the most refined levels of visual acuity and for color vision.
The 1.5 mm diametered macular center, anatomically characterized by a depression in the inner retinal surface, is the anatomical fovea. Its central floor is called the foveola, and is an area 0.35 mm in diameter. In this area, the inner retinal layers (inner nuclear layer and ganglion cell layer) as well as the retinal capillaries are absent, and the axons of the photoreceptors almost immediately bend away from the photoreceptor bodies, nearly parallel to the retinal surface, thereby minimizing light scatter.

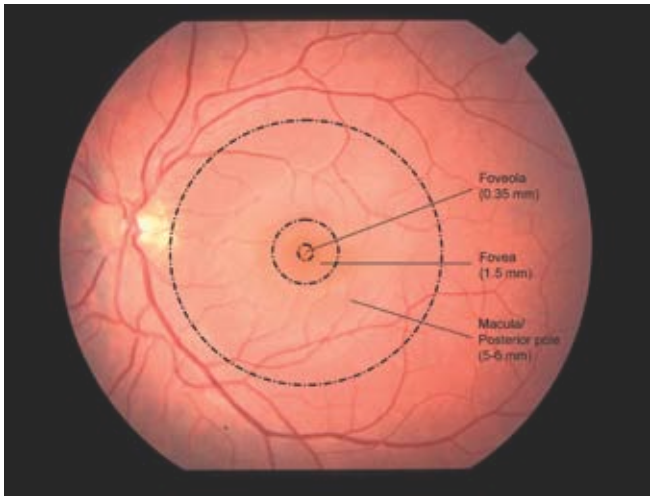

Figure 1.6 The positions of macula, fovea and foveola in the posterior pole of the eye

Photoreceptors

The photoreceptor cells are not equally spread over the retina: the density of photoreceptors is highest in and near the macula as shown in **Figure 1.7**.
Except in the fovea, where only cones are found, the photoreceptor layer contains two types of photoreceptors: the rods and the cones. The rods are responsible for contrast, brightness and motion sense, whereas the cones provide colour vision, fine resolution and spatial resolution. In humans, the rods represent 95% of the photoreceptors, where cones represent only 5%.

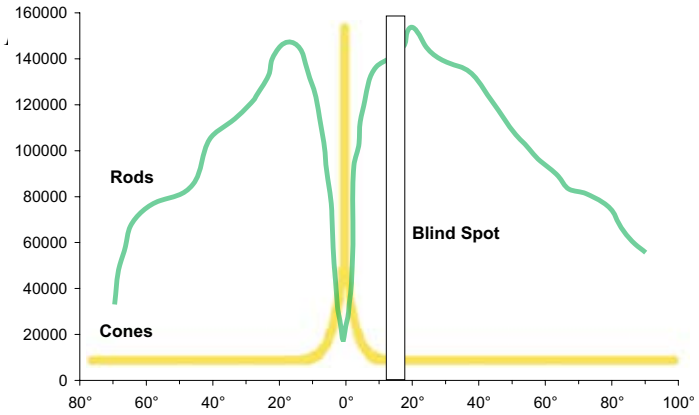

Figure 1.7 Photoreceptor density in the retina

1.2 Retinal detachment

1.2.1 Definition

Retinal detachment is the situation in which fluid is present between the RPE and the photoreceptor layer. This situation can be the result of numerous processes.
Three main types of retinal detachment can be distinguished: the most common rhegmatogenous retinal detachment (RRD), the exudative retinal detachment and the tractional retinal detachment. The latter two types of detachment are sometimes referred to as "nonrhegmatogenous" or "secondary" detachments. Mixed types, however, are possible.

1.2.2 Types of retinal detachment

The term rhegmatogenous retinal detachment is derived from the Greek word "rhegma", which means "rent" or "fissure", referring to the occurrence of a retinal break, through which fluid from the vitreous cavity enters the subretinal space and thereby divides the sensory retina from the RPE. See **Figure 1.8**.

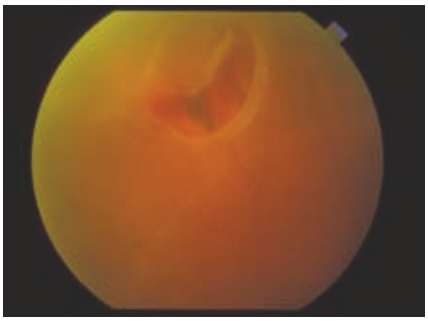

Figure 1.8 Retinal detachment caused by a flap tear

In exudative retinal detachment, the detachment is due to a subretinal fluid producing process, such as an inflammation or a tumor, and a retinal break is not involved.
In a tractional retinal detachment, the traction is caused by pathologic vitreoretinal adhesions, mechanically pulling the retina away from the RPE. Common causes for this type of retinal detachment are proliferative diabetic retinopathy, proliferative vitreoretinopathy (PVR), and penetrating traumata. Other less common causes such as retinopathy of prematurity and proliferative sickle cell retinopathy have been described.
The rhegmatogenous retinal detachment is the most common type of detachment, and the main scope of this thesis.

1.2.3 Epidemiology of RRD

The approximate yearly incidence of RRD is 10 per 100,000 individuals, but this number can vary between different races. The highest reported incidence rate was 17.9 per 100,000 individuals per year in a white population in Minnesota, whereas the lowest reported annual incidence rate was 0.46 per 100,000 individuals, reported in South-African blacks.[2;3] Swedish Caucasians, Chinese, Malay and Indians have incidences of 14.0, 11.6, 7.0 and 3.9 per 100,000 individuals per year respectively.[4;5] Most RRDs occur between 40 and 70 years of age (**Figure 1.9**), and males represent approximately 57-60% of RRD patients.[2;6-8] The overall mean age at diagnosis of RRD is 50 years for men and 59 years for women.[2] For idiopathic, nontraumatic RRD alone, however, the mean age difference between the two sexes is not significant: 53 and 56 years for men and women respectively.[2;7]

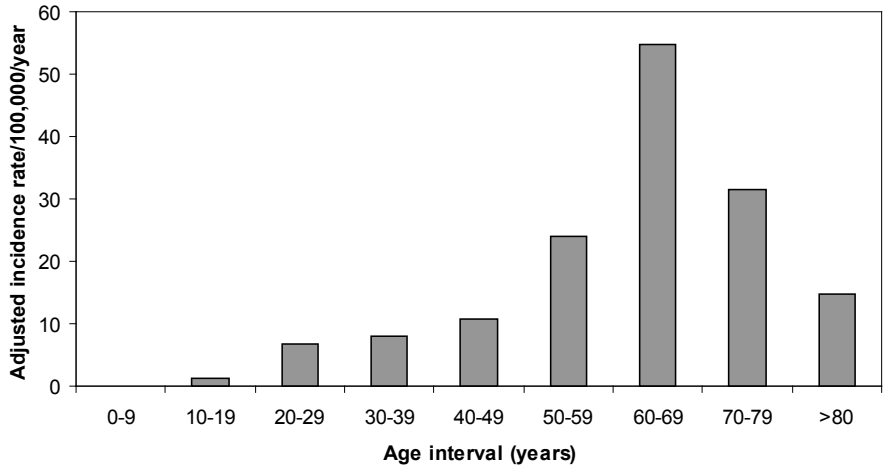

Figure 1.9 Occurrence of RRD in relation to age

Although RRD is slightly more often reported in the right eye than in the left eye, no significant difference was found.[7;9] RRD in the fellow eye -not necessarily simultaneously- has been reported in 4.2-19% of the cases.[2;7;10;11] In the first year after the first RRD, a 2.1% rate has been reported in the fellow eye.[11]

1.2.4 Retinal breaks

RRD is caused by one or more retinal breaks: full-thickness defects in the neurosensory retina, which are designated ‘symptomatic’ if the patient reports photopsia and/or floaters. Asymptomatic retinal breaks are found in 5.8% of patients of 10 years or

older.[12]
Three main types of retinal breaks can be distinguished: flap tears, holes and retinal dialysis.[8]

Flap tears
Flap tears are caused by retinal traction from vitreous adhesions and are often found at the equator of the eye. Approximately half of the flap tears progress to RRD.[13;14] Appearances of tears range from a rarely seen slit-like shape to a more frequently seen complete horseshoe-shape. A tear that circumferentially extends 90 degrees of the eye or more is classified as a giant tear.

Holes
In some cases, the flap is completely torn free from the retina, leading to a hole with an operculum (lid) adhering to the posterior vitreous membrane. These operculated holes tend to occur more posteriorly than flap tears. Because of the loss of traction on the retina after separation of the operculum, they are less apt to cause retinal detachment than the usual tears.[8]
Holes without an operculum are caused by a different mechanism. They are mostly due to gradual atrophy of the retina, and are often found in the equatorial zone or near the ora serrata, in the middle of areas with lattice degeneration, or in association with retinoschisis and chorioretinal atrophy. Rarely, they are caused by ruptured cystoid spaces in peripheral cystoid degeneration. Asymptomatic operculated and atrophic holes are considered quite harmless, and only very rarely lead to RRD.[15]
Macular holes are caused by degeneration or fusion of intraretinal cystoid spaces and also only rarely cause retinal detachment.

Retinal dialysis
A retinal dialysis is a linear, circumferential break found at the ora serrata, and is commonly caused by blunt traumata.

The break types differ in retinal distribution of the breaks: flap tears are most often found in the superior quadrants of the retina, more often in the temporal part. Atrophic retinal holes are also most often found in the temporal quadrants, but as in flap tears, they most often appear in the superior part. Retinal dialyses are most frequently found in the inferior temporal quadrant. Furthermore, most retinal breaks are found near the equator (45%) or anteriorly to the equator (12% at the ora serrata, 28% between the ora and the equator); the rest appears more posteriorly, and only 1% occurs at the macula.[8]

1.2.5 Risk factors
The known risk factors for RRD can be divided in endogenous factors like myopia,

vitreous degeneration and lattice, and acquired factors like trauma and aphakia or pseudophakia.

Myopia

Myopia is found in approximately 56% of all detachment patients. The refractive error in most patients is not lower than –3 diopters, but higher degrees of myopia are associated with higher lifetime risks for RRD. These risks go up to 9.3% for refractive errors of –5 diopters or less, as shown in **Figure 1.10**.[16;17] Furthermore, younger RRD patients tend to have more myopic refractive errors, whereas older patients tend to have refractive errors closer to emmetropia.[16;17]

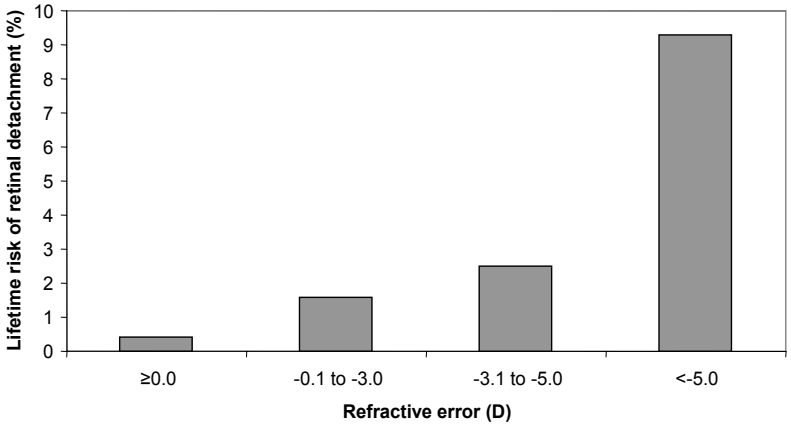

Figure 1.10 Lifetime risks of retinal detachment in relation to refractive error[25]

Vitreous degeneration

Degeneration of the vitreous body is a physiological process seen in aging. (See **Figure 1.11**) A progressive liquefaction process of the vitreous starts in the posterior part, in front of the macular region, and gradually spreads to the anterior parts. This results in a gradual 'fibrous destruction' and formation of optically empty lacunae filled with liquid vitreous in the normally gel-like vitreous body of a young healthy eye. This process is associated with posterior vitreous detachment, which is usually found in eyes with liquefaction of more than half of the vitreous body.[18;19] This usually acute event of posterior vitreous detachment is associated with vitreoretinal traction at the undetached sites, especially in the peripheral retina where the vitreous base is located and where the collagenous adhesion to the retina is strongest. This process leads to retinal breaks in 14% of patients and finally results in RRD in 28% of these patients.[20;21] Posterior vitreous detachment is found in less than 10% of individuals under 60 years of age, in 27% of individuals between 60 and 70, and in 63% of individuals of 70 years and above.[19]

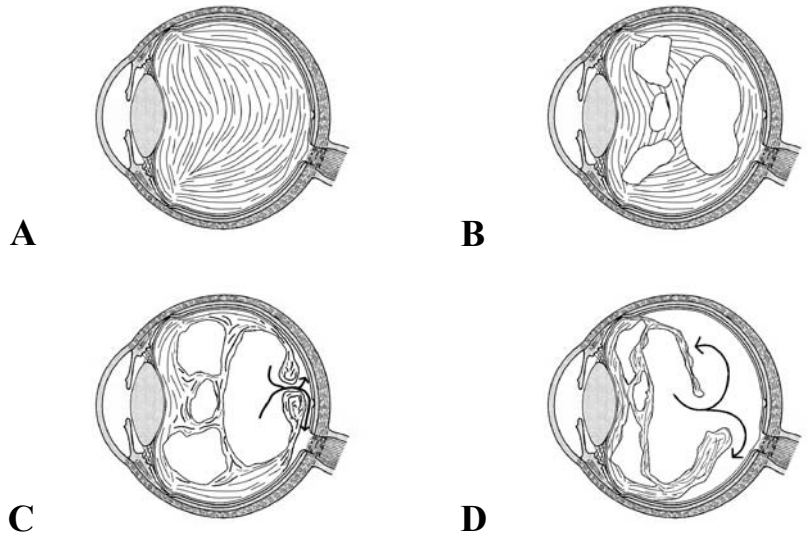

Figure 1.11 Stages of vitreous degeneration
A: The vitreous body has a gel-like consistency and is attached to the complete retina. **B**: The vitreous body liquefies, and starting in the posterior part, lacunae develop. **C**: Further liquefaction causes a lacunar break, enabling the liquid to pass through the break. **D**: A posterior vitreous detachment develops, possibly causing traction on or even a break in the retina.

Lattice degeneration

Lattice degeneration is found in approximately 7-11% of the general population. Most of these people will never develop RRDs and an overall lifetime risk of only 0.5% was estimated.[22] However, about one-third of all phakic RRDs is associated with lattice degeneration; especially younger age groups with lattice and groups with myopia might develop RRD. The presence of lattice degeneration in myopic patients increases the annual incidence of detachment by a factor of approximately 10. The higher the degree of myopia in the presence of lattice, the higher the lifetime risk for RRD. These risks even go up to 35.9% for myopia lower than –5 diopters, whereas emmetropic or hyperopic eyes with lattice degeneration have a lifetime risk of 1.7%.[17]

Trauma

Blunt or perforating trauma-related RRD is seen in 11.6% of RRDs, and has an annual incidence of 1.3 per 100,000 individuals.[2] In an independent study, these numbers are respectively 8% and 0.93 per 100,000 individuals for blunt traumata only.[7] The age of these patients is three to four decades lower than usual in RRD, and this RRD-type is found 5-11 times more often seen in males in comparison to females.[2;7] In juvenile

RRD, trauma is the cause in 44% of patients.[23]

Cataract surgery
The incidence of retinal detachment following cataract surgery ranges from 0.6% to 1.7% during the first postoperative year. After 10 years, the risk is 5.5 times higher in patients who have undergone cataract surgery compared to unoperated patients. In intracapsular cataract extraction the estimated overall incidence of retinal detachment ranges between 1-8.1% after follow-up of between 1 week and 22 years. A higher incidence of retinal detachment after intracapsular cataract extraction in myopic eyes (around 6%) than in non-myopic eyes (1.1-3.6%) was reported. Likewise, retinal detachment was more often reported in aphakic eyes (2.8%) compared to eyes with an implanted intraocular lens (0.2%). In extracapsular cataract extraction, a slightly lower RRD incidence of 0-7.5% (but mostly around 1%) was estimated, which – in comparing studies- was even lower in operations using phacoemulsification (0-3.6%).
The most important factor following cataract extraction probably is the posterior vitreous detachment, which is more commonly seen in patients in whom the lens has been removed. Furthermore, lower concentrations of hyaluronic acid in aphakic eyes compared to phakic fellow eyes in humans, as well as in intracapsular cataract extraction compared to extracapsular cataract extraction in monkeys have been found, suggesting a role of the posterior capsule and/or the hyaluronic acid in maintaining the colloidal structure of the vitreous.[24]

Capsulotomy
Posterior capsulotomy using Nd:YAG laser (which is the short name for the laser that uses a rod of yttrium alumininium garnate containing ions of neodymium as the amplifying medium) treatment has also been associated with an increased risk of retinal detachment of between 0% and 4.1% after a 3 months to 4 year follow-up, and is often performed to resolve opacifications of the posterior lens capsule after extracapsular cataract extraction. This unfortunately hampers accurate estimates of Nd:YAG laser complications isolated from those of extracapsular cataract extraction.[24]

1.2.6 Natural history of RRD

Untreated RRDs invariably lead to marked proliferative vitreoretinopathy, which can provide new traction upon the retina leading to fixated retinal folds, (new) breaks and further detachment. The result can be permanent loss of vision with maximal visual acuities of hand movements. The latter is due to the progression of the RRD, which usually starts with one or more retinal breaks in the periphery of the retina with no or scanty subretinal fluid. Due to gravity forces and the limited subretinal space, it spreads towards the macular region, until finally the retina is detached completely, except around the papilla. (See **Figure 1.12**) Other associated complications of long-

Figure 1.12 Complete retinal detachment.
Note that the papilla centers all folds.

standing RRD are hypotony, cataract, strabismus, low-grade iridocyclitis, rubeosis iridis and phthisis bulbi.[25]

1.2.7 Treatment

The goal of treatment is to achieve reattachment of the neurosensory retina to the retinal pigment epithelium with its underlying choroids, and to prevent the eye from further leakage of subretinal fluid.

Laser coagulation and cryocoagulation

Laser coagulation in cases of clear intra-ocular media and transscleral cryocoagulation in cases of troubled media can be used to create a firm chorioretinal adhesion by inducement of a scarring process. The techniques are used in cases with retinal breaks without clinical retinal detachment to prevent further leakage of subretinal fluid, or in cases where a firm reattachment is needed after surgical realignment of the neurosensory retina and the retinal pigment epithelium. Laser treatment is successful in prevention of progression to RRD in over 95% of cases.[26]

Surgical treatment

In case of an apparent RRD, surgical treatment is needed. Scleral buckling is the most conventional procedure for a local RRD: a silicon buckle is placed around the eye at the site of the retinal break(s), parallel to the corneal limbus. One or more silicon patches ("plombes") are placed between sclera and buckle at the sites of the breaks, thereby causing a local indentation that joins the retinal pigment epithelium and the retinal break. See **Figure 1.13**.

Additional transscleral punction can be used to drain the accumulated subretinal fluid, and a slowly resorbing gas can be injected into the vitreous cavity for additional

Figure 1.13 Scleral buckling with a radial and a limbus-parallel silicon patch

pressure from within the eye and tamponade of the retinal break.
As a second method of treatment for a local RRD, this gas injection is also used without the buckling procedure: a procedure known as pneumatic retinopexy. The procedure is often preceded by cryocoagulation of the defect, and followed by laser treatment. During 5-7 days, patients are requested to position their head in a way that allows the gas bubble to tamponade the retinal break. After one retinopexy, 73% of the detachments is successfully reattached.[27]
Vitrectomy is applied in the more complicated detachments. The vitreous body and the retinal traction are removed using instruments entering the vitreous cavity via three scleral incisions through the pars plana of the ciliary body, enabling repositioning of the retina. Additionally, transscleral punction of accumulated subretinal fluid, laser- or cryotherapy can be used. The vitreous is temporarily replaced by a slowly resorbing gas, or by silicon oil that has to be removed surgically at a later time, to keep the retina in its place. The procedure can be combined with the buckling procedure.
Sometimes, several operations are needed to achieve final reattachment of the retina: a primary reattachment is achieved in about 76-85%.[28] If timely surgical treatment is given, an anatomically good result can be achieved in 90-96% of cases.[28-31]

1.3 Genetics of retinal detachment

Retinal detachment can be part of genetic disorders with or without systemic abnormalities. Due to overlapping symptoms of these disorders, a strict distinction between syndromes is often very difficult, and can sometimes only be made after a certain period of follow-up.
For the clarity of the description below, however, the most important disorders are divided in two groups, according to the presence or absence of non-ocular (systemic) abnormalities. We respectively refer to them as syndromic or nonsyndromic disorders.

1.3.1 Nonsyndromic disorders with retinal detachment

1.3.1.1 High myopia

High or "pathologic" myopia of ≤ -6.00 diopters has a worldwide prevalence ranging from 0.1-2.5%, with exceptions of 9.1% in Singaporean Chinese, 9.6% in Spaniards and 21% in Taiwanese students, although Taiwanese elderly showed the usual prevalence of 2.4%. Furthermore, females had significantly higher rates than males.[32-41] It is primarily caused by increased ocular axial length and may be associated with glaucoma, macular degeneration, cataract and retinal detachment. Other causes are corneal or conical changes of the lens. Furthermore, both environmental factors (such as near work and years of education) and genetic factors (from both population and twin studies) have been implicated as causes for myopic changes in refractive error, but heredity seems to play a more important role in high myopia than in low myopia.[35;42-46]
Autosomal dominant pathologic myopia loci were found at chromosome 18p11.31 (MYP2), chromosome 12q21-q23 (MYP3), chromosome 7q36 (MYP4), and chromosome 17q21-q22 (MYP5).[47-50] The X-linked form of high myopia, Bornholm Eye Disease, showed linkage with chromosome Xq28 (MYP1) and was accompanied by moderate hypoplasia of the optic nerve heads and deuteranopia in all affected males.[51] Strikingly, no genes underlying high myopia have thus far been indentified.

1.3.1.2 Vitreoretinopathies

Wagner disease
Wagner disease was first described in 1938 and is an autosomal dominantly inherited disorder with a usually moderate myopia around –3 to –4 diopters, vitreoretinal abnormalities and a complicated cataract that starts in adolescence and which needs extraction around the fourth decade of life.[52] The eye characteristically shows a very liquid vitreous body that seems optically empty upon slit-lamp biomicroscopy, with an exception of a few threads with very small white dots. A preretinal circular membrane of variable widths -sometimes as thin as a line- at the equator is found in all affected. It is, however, not always complete and the peripheral boundaries are not always

Figure 1.14 Preretinal circular line at the equator in a patient with Wagner disease (arrow)

markedly visible. See **Figure 1.14**.

In an early stadium of the degenerative process of the vitreous body, fibrillar condensations are common, whereas avascular strands and veils are more common among patients older than 30 years of age.
Additional observations are peripheral perivascular hyperpigmented spots in the retina with white vascular sheating, choroidal atrophy, sclerosis of the vessels, pallor of the optic disc or atrophy of the optic nerve and reduced night vision. An abnormal pattern of the central retinal vessels ("situs inversus" or "dragged vessels") was found in half of the patients (**Figure 1.15**).

Fluorescein angiography shows loss of the choriocapillaris and the RPE, with preservation of larger choroidal vessels. Visual fields are concentrically reduced, dark adaptations range from almost normal to strongly reduced values, and scotopic ERG testing reveals subnormal values between 50-100 mV and a prolonged implicit time. Skeletal, cardiovascular or hearing abnormalities are not found.[52-55]

Figure 1.15 Situs inversus of the central retinal vessels in a patient with Wagner disease

Rhegmatogenous retinal detachment, due to flap tears and/or atrophic holes, is described in approximately 15% of patients at an average age of 20 years. Peripheral tractional retinal detachment is described in approximately 35% of patients at an average age of 49 years. Some of these tractional detachments are bilateral.[54] A subtype of Wagner disease with more frequent retinal detachments was described by Jansen, and is sometimes referred to as Jansen syndrome.[56] This subtype was initially thought to be distinct from Wagner disease because retinal detachments had not been described in the original Wagner pedigree, but later follow-up of this pedigree showed retinal detachments after all.[54]
Linkage analysis proved an association with chromosome 5q13-14[57], and later studies that among others included one of the families described by Jansen, achieved further refinement to a 2- to 2.5-cM region of chromosome 5q14.3.[58;59] The responsible gene, however, has not yet been found.

Erosive vitreoretinopathy
The in 1993 first described erosive vitreoretinopathy is inherited in an autosomal dominant fashion, and is characterized by pronounced vitreous syneretic abnormalities, frequently complicated retinal detachments, and a marked progressive pigmentary retinal dystrophy.[60]
The first symptoms in patients are night vision difficulties and progressive constriction of the visual field, mostly starting in young adulthood. Refractive errors vary from emmetropia to –10 diopters, and a nuclear sclerotic cataract requiring extraction of the lens by 40 years of age is common, although this might also be caused by the high incidence of surgery for retinal detachment. Ophthalmoscopy shows marked equatorial RPE thinning or 'erosion' in the course of time enabling visualization of the choroidal vessels, as well as thick rope-like strands, sheets or veils, often with traction at the border of erosive lesions an normal-appearing RPE. Some patients additionally show attenuated, sheathed arterioles with retinal atrophy, as well as dragged disc vessels, an ectopic macula, a positive angle kappa and peripheral retinoschisis.
Retinal detachments can be both tractional and rhegmatogenous, and have an onset under 10 years of age in almost all patients. In about half of the cases they are bilateral. The detachments are often caused by multiple posterior breaks or giant retinal tears, and they are difficult to repair.
Corresponding with the RPE lesions, most eyes show complete or incomplete ring scotoma with progression to the centre. The ERG recordings for rods and cones are reduced, and under light-adapted circumstances, the 30-Hz flicker ERG is prolonged abnormally.
Linkage analysis in the erosive vitreoretinopathy family revealed association with a critical interval of 35 cM that overlaps the Wagner disease locus at chromosome 5q13-q14. Therefore, erosive vitreoretinopathy and Wagner disease might be allelic disorders.[57]

Snowflake vitreoretinal degeneration
Snowflake vitreoretinal degeneration was first described in 1974, and is characterized by early onset cataract, fibrillar degeneration of the vitreous and slowly progressive peripheral retinal abnormalities resembling snowflakes: minute, shiny crystallinelike yellow-white deposits. Rhegmatogenous retinal detachment around the fifth decade of life or later is described in 21% of the patients, due to horseshoe tears or large holes at the equatorial retina. Additionally, corneal guttae, radial perivascular degeneration, vascular sheathing on and near the optic disk and chorioretinal atrophy between round or irregularly shaped peripheral pigment clumps at the posterior margin of the snowflake degeneration have been reported. In later stages the pigmented clumps are more pronounced, and the peripheral vessels seem to whiten and become less visible. Refractive errors range from slight hyperopia to –7 diopters, but the myopia is generally mild.[61;62]

The disease is autosomal dominantly inherited, and has not yet been associated with a certain chromosome. Linkage with the Wagner locus and several collagen genes associated with vitreoretinal degeneration (*COL2A1*, *COL11A1*, *COL9A1*, *COL9A2* and *COL9A3*) has been excluded.[62]

Familial exudative vitreoretinopathy
Familial exudative vitreoretinopathy, first described in 1969, is characterized by bilateral deficient vascularisation of the peripheral retina that can be visualized with fluorescein angiography. The phenotype, however, can be variable: from mild with retinal traction that straightens the temporal retinal vasculature and moderate with distortion and displacement of the macula and the vessels around the optic disk, to severe with traction leading to retinal folds and detachment.[63;64] The retinal detachment usually occurs in the second or third decades of life, is seen in 20-32% of patients and can be tractional, exudative or rhegmatogenous in origin. The latter, however, is the most frequent.[65;66] Other findings in patients are peripheral snowflake vitreous changes, and areas of normal retina except for the whiter aspect, appearing without (or upon) indentation called “white without (or with) pressure”. These “white without pressure” areas are only significant if a giant tear in the fellow eye has appeared: they may then be associated with an increased risk of retinal breaks.[67] Although familial exudative vitreoretinopathy generally occurs bilaterally, the severity of the disease can be asymmetric.
In autosomal dominant familial exudative vitreoretinopathy, which is the most common type of inheritance[66;68], two genes at the same locus EVR1 at chromosome 11q13-q23 have been identified: the *FZD4* gene and the *LRP5* gene.[69;70] *FZD4* encodes the Wnt receptor frizzled-4, and *LRP5* encodes low-density-lipoprotein receptor-related protein 5, a Wnt co-receptor. An additional locus, EVR3, for autosomal dominant familial exudative vitreoretinopathy has been found on chromosome 11p12-p13, but the gene remains unidentified.[71] In the X-linked form of the disorder, mutations in the Norrie disease gene (*ND*) at the EVR2 locus at chromosome Xp11.3-p11.4 have been

described.[72;73] Autosomal recessive inheritance of the disorder has been described in families, but the responsible loci has not yet been identified.[74;75]

1.3.1.3 Other disorders

X-linked juvenile retinoschisis

The first description of X-linked juvenile retinoschisis dates from 1898. The disorder usually presents with a deteriorated vision in childhood and shows a characteristic maculopathy with tiny superficial cystoid spaces in a stellate pattern surrounding the fovea, which can coalesce and in time will disappear and leave only a non-specific atrophic lesion in both macula and underlying RPE. In approximately 50% of patients a thin retinoschisis is present, consisting of the internal limiting membrane and the retinal nerve fibre layer. If defects develop in the layers of the schisis cavity or if a full-thickness break develops in other areas, an RRD can develop. Retinal detachments are described in 0-23% of patients, mostly in the first two decades of life.[76-78] Only few of these detachments are exudative.[79] Another common complication of the disorder is a vitreous hemorrhage, due to ruptured retinal vessels in areas of retinoschisis. Most patients are hyperopic or emmetropic, but also myopic patients have been described.[18;76] The disease was caused by mutations in the *RS1* gene at Xp22.13, coding for retinoschisin. This protein is thought to be excreted by the photoreceptors, and speculated to be involved in either cell-cell interaction or adhesion, thereby contributing to the maintenance of the cellular architecture of the retina.[80;81]

Nonsyndromic Congenital Retinal Nonattachment

This disorder was first described in 1938, and inherits both autosomal dominantly and autosomal recessively. As the name suggests, a congenital unattached retina is seen in children. Other described ocular abnormalities related to the disorder are congenital light insensitivity, massive retrolental masse, shallow anterior chamber, microphthalmia and nystagmus. The responsible gene has not yet been identified, but linkage with chromosome 10q21 has been found.[82;83]

1.3.2 Syndromic disorders with retinal detachment

1.3.2.1 Stickler syndrome

The autosomal dominant Stickler syndrome was first described in 1965.[84] The disorder comprises ocular abnormalities, hearing loss and skeletal abnormalities, although a non-ocular form of Stickler syndrome was also described. Ocular Stickler syndrome is diagnosed if a congenital vitreous anomaly consisting of either a retrolental membrane (type I) or irregular thickened ('beaded') fibrils (type II) exists, in combination with at least three of the following: (usually stable) myopia with onset before 6 years of age (a), rhegmatogenous retinal detachment or paravascular pigmented lattice degeneration (b), joint hypermobility with abnormal Beighton score, either with or without radiological evidence of joint degeneration (c), audiometric confirmation of

sensorineural hearing loss (d), and midline clefting (e). Other abnormalities such as cataract, midfacial hypoplasia, slender extremities, long fingers with normal body height, and mitral valve prolapse were also associated with the syndrome.[85-87]
The RRDs in Stickler syndrome are mostly found in children, adolescents or young adults, and appear in approximately 50% of patients with Stickler and are often bilateral.[88] They usually result from giant retinal tears or multiple breaks in retinal areas with thinning of the RPE and lattice degeneration.[85;89]
Phenotypic variation within and between families is well known, although the vitreous abnormalities seem consistent in families and seem indicative for the causative genetic defects: the membranous vitreous anomaly is associated with mutations in the *COL2A1* gene on chromosome 12q13.11-q13.2, whereas the beaded fibrillar anomaly is associated with mutations in the *COL11A1* gene on chromosome 1p21.[85] However, the value of these observations and whether they always correctly indicate the causative gene is still subject of discussion, because a Stickler family with a membranous vitreous anomaly showed linkage with *COL11A1*, two Stickler families with a fibrillar anomaly showed no linkage with *COL11A1*, and a third afibrillar anomaly was described in a family with a *COL2A1* mutation. Finally, only a minority of patients from a family with a known Stickler mutation in the *COL2A1* gene showed a type I vitreous.[90-94]
Non-ocular Stickler syndrome has been associated with mutations in the *COL11A2* gene on chromosome 6p21.3, and a fourth locus for Stickler syndrome is suspected, because in some families mutations in all three known genes have been excluded.[91;95-97]

1.3.2.2 Marshall syndrome

Whether Marshall syndrome is a distinct clinical entity from Stickler syndrome is still an unresolved issue; the facial appearance in the two syndromes seems to make the difference.[98] The original family described in 1958 showed autosomal dominant inheritance of cataracts, high myopia, abnormal vitreous, pronounced midfacial hypoplasia and congenital deafness.[99] Additional hypertelorism as a specific feature of the disorder was described[100], and short stature, long philtrum, short nose and anteverted nostrils were associated with the syndrome.[101] Whether ectodermal dysplasia, as suggested in the original family, is a feature of the condition is also still debated. However, the hair in patients of the original family was normal, and hypodontia and hypohidrosis were not strongly and convincingly present, although the latter was estimated 25% lower than normal in patients.[99]
Rhegmatogenous retinal detachments were suggested to occur less frequently in Marshall syndrome than in Stickler syndrome.[98;102]
As in Stickler syndrome, mutations in the *COL11A1* gene were found; splice site mutations in a 54-bp exon in the C-terminal end of the *COL11A1* gene were associated with the Marshall syndrome phenotype, while other *COL11A1* mutations caused a phenotype with features of both Marshall and Stickler syndrome.[101;103]

1.3.2.3 Kniest dysplasia

Kniest dysplasia, first described in 1952, is a generalized disorder of connective tissue and is characterized by short-trunk dwarfism, kyphoscoliosis, prominent joints with restricted mobility, midface hypoplasia, cleft palate, hearing loss, combined with ocular abnormalities as prominent eyes, high myopia with vitreous liquefaction and syneresis and/or retinal detachment.[104-107] Cartilage biopsies have disclosed a "Swiss cheese" appearance: empty spaces in the cartilage matrix, which is loosely woven and contains large chondrocytes.[108]
The retinal detachment develops in almost 50% of the patients, often before 6 years of age.[18] The retinal defects can present as multiple tears in the periphery, as giant tears or as dialysis at the ora serrata.[18;106]
So far, all mutations responsible for the disease have been found in the *COL2A1* gene. It has been suggested that Kniest dysplasia results from shorter type II collagen monomers, and that alteration of a specific COL2A1 domain, which may span from exons 12 to 24, leads to the phenotype.[109]

1.3.2.4 Marfan syndrome

The Marfan syndrome, first described in 1896, is a usually autosomal dominant inherited disorder with a variable expression. The combination of cardiovascular abnormalities (such as dissection or dilatation of the ascending aorta and aortic regurgitation), the skeleton (such as long and thin limbs, arachnodactyly, pectus deformity, scoliosis and laxity of the joints) and the eye (such as dislocated lenses, axial myopia, flat cornea, hypoplastic iris or ciliary muscle, liquefaction of the vitreous, peripheral retinal changes, lattice degeneration and retinal holes) is classic. Lately, lumbosacral dural ectasia has become one of the major criteria. Additional abnormalities can be found in the pulmonary system or skin.[110-113]
RRDs were seen in 3.5-25% of Marfan syndrome patients and were mostly caused by multiple retinal tears, but also by holes or oral dialysis.[114-121] Bilateral detachments were seen in 34.5% of patients.[121]
The syndrome is caused by mutations in the fibrillin (*FBN-1*) gene, residing at chromosome 15q21.1.[122-124]

1.3.2.5 Knobloch syndrome

Knobloch syndrome is a rare autosomal recessive disorder, first described in 1971, and characterized by high myopia, macular abnormalities, vitreoretinal degeneration with retinal detachment, macular abnormalities and occipital encephalocele. The expression of the syndrome is variable within or between families, but shows a 100% penetrance.[125-128] Other abnormalities in single families comprise lens subluxation, cataracts, flat nasal bridge, midface hypoplasia, bilateral epicanthic folds, hyperextensible joints, right lung hypoplasia with anomalous pulmonary return, cardiac dextroversion, unilateral duplicated renal collecting system and unusual palmar creases.[125-129]
Rhegmatogenous retinal detachments in the original family were seen under ten years

of age, were often bilateral and were caused by multiple tears, some with rolled edges, or by a giant tear.[130]
Homozygous mutations have been found in the *COL18A1* gene at chromosome 21q22.3, but another locus is suspected, as in some families and single patients mutations in the *COL18A1* gene have been excluded.[131;132]

1.3.2.6 Other disorders

In Norrie disease, congenital degenerative and proliferative changes in the neuroretina and vitreous lead to a disorganized neuroretina, vitreoretinal haemorrhages and leukocoria due to retrolental fibrovascular masses, retinal folding and detachment. Finally, this process results in progressive ocular atrophy and blindness. Mental retardation and sensorineural deafness develop in one-third of all patients, and a wide inter- and intrafamilial variety of severity and clinical course of the disorder is known.[18;133] The inheritance is X-chromosomal, and mutations in the *ND* gene at chromosome Xp11.4 are causative for the disease.[133]
Retinal detachments are also described in diabetes mellitus, in which genetic predisposition is suspected and considerable heterogeneity exists, making the determination of the exact modes of inheritance difficult and unclear. These retinal detachments can be both tractional and rhegmatogenous, which is also the case in sickling hemoglobinopathies.[18;134] RRDs were rarely reported in patients with homocystinuria, which inherits in an autosomal recessive way, and in patients with Ehlers-Danlos syndrome, of which many subtypes exist, and which can be inherited either autosomal dominantly, autosomal recessively or X-chromosomal.[18;115;135]

1.4 Aims of this thesis

Although knowledge about the inheritance of syndromes with retinal detachment has strongly grown during the last decades, little is known about nonsyndromic forms of inherited retinal detachment. During the last few decades, the knowledge about and experience in techniques for surgical treatment and prevention of retinal detachment has also expanded enormously, possibly enabling prevention or early treatment in patients at risk by inheritance.
We tried to investigate the magnitude of the genetic risk of nonsyndromic RRD and the risk for relatives of patients with RRD, which is described in **Chapter 2**.
To investigate possible differences in presentation and in surgical prognosis with regular RRDs, we have clinically studied two large families with autosomal dominant nonsyndromic retinal detachments, as described in **Chapter 3**.
To identify the gene(s) involved in retinal detachment, we studied these two large families with autosomal dominant nonsyndromic retinal detachments. Linkage analysis showed linkage of the disorder with a chromosomal region on chromosome 12 containing the *COL2A1* gene in both families. This gene indeed showed a mutation in one of these families, as described in **Chapter 4**.
To investigate if the *COL2A1* gene was involved in other families with retinal detachments, and, if so, to what extent, we studied smaller multiplex families with at least three retinal detachments in at least two generations. This study is described in **Chapter 5**.

References

1. Justice J, Lehmann RP. Cilioretinal Arteries- A Study Based on Review of Stereo Fundus Photographs and Fluorescein Angiographic Findings. *Arch.Ophthalmol.* 1976;94:1355-8.
2. Rowe JA, Erie JC, Baratz KH, Hodge DO, Gray DT, Butterfield L et al. Retinal detachment in Olmsted County, Minnesota, 1976 through 1995. *Ophthalmology* 1999;106:154-9.
3. Peters AL. Retinal-Detachment in Black South-Africans. *S.Afr.Med.J.* 1995;85:158-9.
4. Algvere PV, Jahnberg P, Textorius O. The Swedish Retinal Detachment Register. I. A database for epidemiological and clinical studies. *Graefes Arch.Clin.Exp. Ophthalmol.* 1999;237:137-44.
5. Wong TY, Tielsch JM, Schein OD. Racial difference in the incidence of retinal detachment in Singapore. *Arch.Ophthalmol.* 1999;117:379-83.
6. Mowatt L, Shun-Shin G, Price N. Ethnic differences in the demand incidence of retinal detachments in two districts in the West Midlands. *Eye* 2003;17:63-70.
7. Li XX, Wang JZ, Zhao JL, Li Z, Chen L, Wang H et al. Incidence and epidemiological characteristics of rhegmatogenous retinal detachment in Beijing, China. *Ophthalmology* 2003;110:2413-7.
8. Hilton GF, McLean EB, Chuang EL. *Retinal Detachment*. 1989 Fifth ed. San Francisco, California: American Academy of Ophthalmology, 1953: 1-227.
9. Ivanisevic M, Bojic L, Eterovic D. Epidemiological study of nontraumatic phakic rhegmatogenous retinal detachment. *Ophthalmic Res.* 2000;32:237-9.
10. Schepens CL, Marden D. Data on the natural history of retinal detachment. Further characterization of certain unilateral nontraumatic cases. *Am.J.Ophthalmol.* 1966;61:213-26.
11. Sasaki K, Ideta H, Yonemoto J, Tanaka S, Hirose A, Oka C. Epidemiologic characteristics of rhegmatogenous retinal detachment in Kumamoto, Japan. *Graefes Arch.Clin.Exp.Ophthalmol.* 1995;233:772-6.
12. Byer NE. Subclinical retinal detachment resulting from asymptomatic retinal breaks: prognosis for progression and regression. *Ophthalmology* 2001;108:1499-503.
13. Shea M, Davis MD, Kamel I. Retinal breaks without detachment, treated and untreated. *Mod.Probl.Ophthalmol.* 1974;12:97-102.
14. Davis MD. Natural history of retinal breaks without detachment. *Arch.Ophthalmol.* 1974;92:183-94.
15. Byer NE. What happens to untreated asymptomatic retinal breaks, and are they affected by posterior vitreous detachment? *Ophthalmology* 1998;105:1045-9.

16. Yannuzzi LA, Sorenson JA, Sobel RS, Daly JR, Derosa JT, Seddon JM et al. Risk-Factors for Idiopathic Rhegmatogenous Retinal-Detachment. *Am.J.Epidemiol.* 1993;137:749-57.

17. Burton TC. The influence of refractive error and lattice degeneration on the incidence of retinal detachment. *Trans.Am.Ophthalmol.Soc.* 1989;87:143-55.

18. Michels RG, Wilkinson CP, Rice TA. *Retinal detachment*. St. Louis, Missouri: The C.V. Mosby Company, 1990: 1-1138.

19. Foos RY, Wheeler NC. Vitreoretinal Juncture - Synchysis Senilis and Posterior Vitreous Detachment. *Ophthalmology* 1982;89:1502-12.

20. Novak MA, Welch RB. Complications of acute symptomatic posterior vitreous detachment. *Am.J.Ophthalmol.* 1984;97:308-14.

21. Byer NE. Natural history of posterior vitreous detachment with early management as the premier line of defense against retinal detachment. *Ophthalmology* 1994;101:1503-13.

22. Byer NE. Changes in and prognosis of lattice degeneration of the retina. *Trans. Am.Acad.Ophthalmol.Otolaryngol.* 1974;78:OP114-OP125.

23. Butler TKH, Kiel AW, Orr GM. Anatomical and visual outcome of retinal detachment surgery in children. *Br.J.Ophthalmol.* 2001;85:1437-9.

24. Lois N, Wong D. Pseudophakic retinal detachment. *Surv.Ophthalmol.* 2003;48:467-87.

25. Ivanisevic M. The natural history of untreated rhegmatogenous retinal detachment. *Ophthalmologica* 1997;211:90-2.

26. La Heij EC, Hendrikse F. [Retinal detachments and retinal surgery]. *Ned.Tijdschr. Geneeskd.* 1999;143:781-5.

27. Tornambe PE, Hilton GF. Pneumatic retinopexy. A multicenter randomized controlled clinical trial comparing pneumatic retinopexy with scleral buckling. The Retinal Detachment Study Group. *Ophthalmology* 1989;96:772-83.

28. Ah-Fat FG, Sharma MC, Majid MA, McGalliard JN, Wong D. Trends in vitreoretinal surgery at a tertiary referral centre: 1987 to 1996. *Br.J.Ophthalmol.* 1999;83:396-8.

29. The repair of rhegmatogenous retinal detachments. American Academy of Ophthalmology. *Ophthalmology* 1990;97:1562-72.

30. Ross WH, Kozy DW. Visual recovery in macula-off rhegmatogenous retinal detachments. *Ophthalmology* 1998;105:2149-53.

31. Minihan M, Tanner V, Williamson TH. Primary rhegmatogenous retinal detachment: 20 years of change. *Br.J.Ophthalmol.* 2001;85:546-8.

32. Fuchs A. Frequency of Myopia Gravis. *Am.J.Ophthalmol.* 1960;49:1418-9.

33. Curtin BJ. *The myopias: basic science and clinical management*. Philadelphia: Harper & Row, 1985.

34. Lithander J. Prevalence of myopia in school children in the Sultanate of Oman: A nation-wide study of 6292 randomly selected children. *Acta Ophthalmol.Scand.* 1999;77:306-9.

35. Guggenheim JA, Kirov G, Hodson SA. The heritability of high myopia: a reanalysis of Goldschmidt's data. *J.Med.Genet.* 2000;37:227-31.

36. Villarreal MG, Ohlsson J, Abrahamsson M, Sjostrom A, Sjostrand J. Myopisation: the refractive tendency in teenagers. Prevalence of myopia among young teenagers in Sweden. *Acta Ophthalmol.Scand.* 2000;78:177-81.

37. Wong TY, Foster PJ, Hee J, Ng TP, Tielsch JM, Chew SJ et al. Prevalence and risk factors for refractive errors in adult Chinese in Singapore. *Invest.Ophthalmol.Vis.Sci.* 2000;41:2486-94.

38. Villarreal GM, Ohlsson J, Cavazos H, Abrahamsson M, Mohamed JH. Prevalence of myopia among 12- to 13-year-old schoolchildren in Northern Mexico. *Optom.Vis. Sci.* 2003;80:369-73.

39. Saw SM, Gazzard G, Koh D, Farook M, Widjaja D, Lee J et al. Prevalence rates of refractive errors in Sumatra, Indonesia. *Invest.Ophthalmol.Vis.Sci.* 2002;43:3174-80.

40. Lin LLK, Shih YF, Hsiao CK, Chen CJ. Prevalence of myopia in Taiwanese schoolchildren: 1983 to 2000. *Ann.Acad.Med.Singapore* 2004;33:27-33.

41. Cheng CY, Hsu WM, Liu JH, Tsai SY, Chou P. Refractive errors in an elderly Chinese population in Taiwan: The Shihpai eye study. *Invest.Ophthalmol.Vis.Sci.* 2003;44:4630-8.

42. Goldschmidt E. On the etiology of myopia. An epidemiological study. *Acta Ophthalmol.(Copenh)* 1968;Suppl 98:1-172.

43. Angle J, Wissmann DA. The epidemiology of myopia. *Am.J.Epidemiol.* 1980;111:220-8.

44. Mohan M, Pakrasi S, Garg SP. The role of environmental factors and hereditary predisposition in the causation of low myopia. *Acta Ophthalmol.Suppl.* 1988;185:54-7.

45. Kinge B, Midelfart A, Jacobsen G, Rystad J. The influence of near-work on development of myopia among university students. A three-year longitudinal study among engineering students in Norway. *Acta Ophthalmol.Scand.* 2000;78:26-9.

46. Lyhne N, Sjolie AK, Kyvik KO, Green A. The importance of genes and environment for ocular refraction and its determiners: a population based study among 20-45 year old twins. *Br.J.Ophthalmol.* 2001;85:1470-6.

47. Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS et al. Evidence that a locus for familial high myopia maps to chromosome 18p. *Am.J.Hum. Genet.* 1998;63:109-19.

48. Young TL, Ronan SM, Alvear AB, Wildenberg SC, Oetting WS, Atwood LD et al. A second locus for familial high myopia maps to chromosome 12q. *Am.J.Hum.Genet.* 1998;63:1419-24.

49. Naiglin L, Gazagne C, Dallongeville F, Thalamas C, Idder A, Rascol O et al. A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. *J.Med.Genet.* 2002;39:118-24.

50. Paluru P, Ronan SM, Heon E, Devoto M, Wildenberg SC, Scavello G et al. New locus for autosomal dominant high myopia maps to the long arm of chromosome 17. *Invest.Ophthalmol.Vis.Sci.* 2003;44:1830-6.

51. Schwartz M, Haim M, Skarsholm D. X-linked myopia: Bornholm eye disease. Linkage to DNA markers on the distal part of Xq. *Clin.Genet.* 1990;38:281-6.

52. Wagner H. Ein bisher unbekanntes Erbleiden des Auges (Degeneratio hyaloideo-retinalis hereditaria), beobachtet im Kanton Zürich. *Klin.Monatsbl.Augenheilkd.* 1938;100:840-57.

53. Böhringer HR, Dieterle P, Landolt E. Zur Klinik und Pathologie der Degeneratio hyaloideo-retinalis hereditaria (Wagner). *Opthalmologica* 1960;139:330-8.

54. Graemiger RA, Niemeyer G, Schneeberger SA, Messmer EP. Wagner vitreoretinal degeneration. Follow-up of the original pedigree. *Ophthalmology* 1995;102:1830-9.

55. Maumenee IH, Stoll HU, Mets MB. The Wagner syndrome versus hereditary arthroophthalmopathy. *Trans.Am.Ophthalmol.Soc.* 1982;80:349-65.

56. Jansen LMAA. Degeneratio hyaloideo-retinalis hereditaria. *Opthalmologica* 1962;144:458-64.

57. Brown DM, Graemiger RA, Hergersberg M, Schinzel A, Messmer EP, Niemeyer G et al. Genetic linkage of Wagner disease and erosive vitreoretinopathy to chromosome 5q13-14. *Arch.Ophthalmol.* 1995;113:671-5.

58. Zech JC, Morle L, Vincent P, Alloisio N, Bozon M, Gonnet C et al. Wagner vitreoretinal degeneration with genetic linkage refinement on chromosome 5q13-q14. *Graefes Arch.Clin.Exp.Ophthalmol.* 1999;237:387-93.

59. Perveen R, Hart-Holden N, Dixon MJ, Wiszniewski W, Fryer AE, Brunner HG et al. Refined genetic and physical localization of the Wagner disease (WGN1) locus and the genes CRTL1 and CSPG2 to a 2- to 2.5-cM region of chromosome 5q14.3. *Genomics* 1999;57:219-26.

60. Brown DM, Kimura AE, Weingeist TA, Stone EM. Erosive vitreoretinopathy. A new clinical entity. *Ophthalmology* 1994;101:694-704.

61. Hirose T, Lee KY, Schepens CL. Snowflake Degeneration in Hereditary Vitreoretinal Degeneration. *Am.J.Ophthalmol.* 1974;77:143-53.

62. Lee MM, Ritter R, Hirose T, Vu CD, Edwards AO. Snowflake vitreoretinal degeneration - Follow-up of the original family. *Ophthalmology* 2003;110:2418-26.

63. Criswick VG, Schepens CL. Familial Exudative Vitreoretinopathy. *Am.J.Ophthalmol.* 1969;68:578-&.

64. Bamashmus MA, Downey LM, Inglehearn CF, Gupta SR, Mansfield DC. Genetic heterogeneity in familial exudative vitreoretinopathy; exclusion of the EVR1 locus on chromosome 11q in a large autosomal dominant pedigree. *Br.J.Ophthalmol.* 2000;84:358-63.

65. Van Nouhuys CE. Dominant Exudative Vitreoretinopathy and Other Vascular Developmental Disorders of the Peripheral Retina. *Doc.Ophthalmol.* 1982;54:1-415.

66. Kondo H, Ohno K, Tahira T, Hayashi H, Oshima K, Hayashi K. Delineation of the critical interval for the familial exudative vitreoretinopathy gene by linkage and haplotype analysis. *Hum.Genet.* 2001;108:368-75.

67. Freeman HM. Fellow eyes of giant retinal breaks. *Trans.Am.Ophthalmol.Soc.* 1978;76:343-82.

68. Shastry BS, Hejtmancik JF, Hiraoka M, Ibaraki N, Okubo Y, Okubo A et al. Linkage and candidate gene analysis of autosomal-dominant familial exudative vitreoretinopathy. *Clin.Genet.* 2000;58:329-32.

69. Robitaille J, MacDonald MLE, Kaykas A, Sheldahl LC, Zeisler J, Dube MP et al. Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. *Nat.Genet.* 2002;32:326-30.

70. Toomes C, Bottomley HM, Jackson RM, Towns KV, Scott S, Mackey DA et al. Mutations in LRP5 or FZD4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. *Am.J.Hum.Genet.* 2004;74:721-30.

71. Downey LM, Keen TJ, Roberts E, Mansfield DC, Bamashmus M, Inglehearn CF. A new locus for autosomal dominant familial exudative vitreoretinopathy maps to chromosome 11p12-13. *Am.J.Hum.Genet.* 2001;68:778-81.

72. Chen ZY, Battinelli EM, Fielder A, Bundey S, Sims K, Breakefield XO et al. A Mutation in the Norrie Disease Gene (Ndp) Associated with X-Linked Familial Exudative Vitreoretinopathy. *Nat.Genet.* 1993;5:180-3.

73. Shastry BS, Hejtmancik JF, Trese MT. Identification of novel missense mutations in Norrie disease gene associated with one X-linked and four sporadic cases of familial exudative vitreoretinopathy. *Hum.Mutat.* 1997;9:396-401.

74. de Crecchio G, Simonelli F, Nunziata G, Mazzeo S, Greco GM, Rinaldi E et al. Autosomal recessive familial exudative vitreoretinopathy: evidence for genetic heterogeneity. *Clin.Genet.* 1998;54:315-20.

75. Shastry BS, Trese MT. Familial exudative vitreoretinopathy: further evidence for genetic heterogeneity. *Am.J.Med.Genet.* 1997;69:217-8.

76. Deutman AF. *The hereditary dystrophies of the posterior pole of the eye*. Assen, The Netherlands: Koninklijke van Gorcum & Comp. N.V., 1971: 1-484.

77. Kellner U, Brummer S, Foerster MH, Wessing A. X-Linked Congenital Retinoschisis. *Graefes Arch.Clin.Exp.Ophthalmol.* 1990;228:432-7.

78. George NDL, Yates JRW, Moore AT. Clinical features in affected males with X-linked retinoschisis. *Arch.Ophthalmol.* 1996;114:274-80.

79. Fong DS, Frederick AR, Blumenkranz MS, Walton DS. Exudative retinal detachment in X-linked retinoschisis. *Ophthalmic Surg.Lasers.* 1998;29:332-5.

80. Sauer CG, Gehrig A, WarnekeWittstock R, Marquardt A, Ewing CC, Gibson A et al. Positional cloning of the gene associated with X-linked juvenile retinoschisis. *Nat. Genet.* 1997;17:164-70.

81. Grayson C, Reid SNM, Ellis JA, Rutherford A, Sowden JC, Yates JRW et al. Retinoschisin, the X-linked retinoschisis protein, is a secreted photoreceptor protein, and is expressed and released by Weri-Rb1 cells. *Hum.Mol.Genet.* 2000;9:1873-9.

82. Ghiasvand NM, Kanis AB, Helms C, Sheffield VC, Stone EM, Donis-Keller H. Nonsyndromic congenital retinal nonattachment gene maps to human chromosome band 10q21. *Am.J.Med.Genet.* 2000;90:165-8.

83. Ghiasvand NM, Fleming TP, Helms C, Avisa A, Donis-Keller H. Genetic fine mapping of the gene for nonsyndromic congenital retinal nonattachment. *Am.J.Med. Genet.* 2000;92:220-3.

84. Stickler GB. Hereditary progressive arthro-ophthalmopathy. *Mayo Clin.Proc.* 1965;40:433-55.

85. Snead MP, Yates JR. Clinical and Molecular genetics of Stickler syndrome. *J.Med. Genet.* 1999;36:353-9.

86. Beals RK. Hereditary Arthro-Ophthalmopathy (Stickler Syndrome) - Report of A Kindred with Protrusio Acetabuli. *Clin.Orthop.Relat.Res.* 1977;32-5.

87. Liberfarb RM, Goldblatt A. Prevalence of mitral-valve prolapse in the Stickler syndrome. *Am.J.Med.Genet.* 1986;24:387-92.

88. Watanabe Y, Ueda M, AdachiUsami E. Retinal detachment in identical twins with Stickler syndrome type 1. *Br.J.Ophthalmol.* 1996;80:976-81.

89. Nowak CB. Genetics and hearing loss: A review of Stickler syndrome. *J.Commun. Disord.* 1998;31:437-54.

90. Parentin F, Sangalli A, Mottes M, Perissutti P. Stickler syndrome and vitreoretinal degeneration: correlation between locus mutation and vitreous phenotype. Apropos of a case. *Graefes Arch.Clin.Exp.Ophthalmol.* 2001;239:316-9.

91. Wilkin DJ, Mortier GR, Johnson CL, Jones MC, De Paepe A, Shohat M et al. Correlation of linkage data with phenotype in eight families with Stickler syndrome. *Am.J.Med.Genet.* 1998;80:121-7.

92. McLeod D, Black GC, Bishop PN. Vitreous phenotype: genotype correlation in Stickler syndrome. *Graefes Arch.Clin.Exp.Ophthalmol.* 2002;240:63-5.

93. Go SL, Maugeri A, Mulder JJS, van Driel MA, Cremers FPM, Hoyng CB. Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the COL2A1 gene. *Invest.Ophthalmol.Vis.Sci.* 2003;44:4035-43.

94. Richards AJ, Baguley DM, Yates JR, Lane C, Nicol M, Harper PS et al. Variation in the vitreous phenotype of Stickler syndrome can be caused by different amino acid substitutions in the X position of the type II collagen Gly-X-Y triple helix. *Am.J.Hum. Genet.* 2000;67:1083-94.

95. Brunner HG, van Beersum SE, Warman ML, Olsen BR, Ropers HH, Mariman EC. A Stickler syndrome gene is linked to chromosome 6 near the COL11A2 gene. *Hum. Mol.Genet.* 1994;3:1561-4.

96. Martin S, Richards AJ, Yates JR, Scott JD, Pope M, Snead MP. Stickler syndrome: further mutations in COL11A1 and evidence for additional locus heterogeneity. *Eur. J.Hum.Genet.* 1999;7:807-14.

97. Sirko-Osadsa DA, Murray MA, Scott JA, Lavery MA, Warman ML, Robin NH. Stickler syndrome without eye involvement is caused by mutations in COL11A2, the gene encoding the alpha2(XI) chain of type XI collagen. *J.Pediatr.* 1998;132:368-71.

98. Ayme S, Preus M. The Marshall and Stickler Syndromes - Objective Rejection of Lumping. *J.Med.Genet.* 1984;21:34-8.

99. Marshall D. Ectodermal Dysplasia - Report of Kindred with Ocular Abnormalities and Hearing Defect. *Am.J.Ophthalmol.* 1958;45:143-56.

100. Shanske AL, Bogdanow A, Shprintzen RJ, Marion RW. The Marshall syndrome: Report of a new family and review of the literature. *Am.J.Med.Genet.* 1997;70:52-7.

101. Griffith AJ, Sprunger LK, Sirko-Osadsa DA, Tiller GE, Meisler MH, Warman ML. Marshall syndrome associated with a splicing defect at the COL11A1 locus. *Am.J.Hum.Genet.* 1998;62:816-23.

102. Stratton RF, Lee B, Ramirez F. Marshall Syndrome. *Am.J.Med.Genet.* 1991;41:35-8.

103. Annunen S, Korkko J, Czarny M, Warman ML, Brunner HG, Kaariainen H et al. Splicing mutations of 54-bp exons in the COL11A1 gene cause Marshall syndrome, but other mutations cause overlapping Marshall/Stickler phenotypes. *Am.J.Hum. Genet.* 1999;65:974-83.

104. Lachman RS, Rimoin DL, Hollister DW, Dorst JP, Siggers DC, Mcalister W et al. Kniest Syndrome. *Am.J.Roentgenol.Radium Ther.Nucl.Med.* 1975;123:805-14.

105. Gilbert-Barnes E, Langer LO, Jr., Opitz JM, Laxova R, Sotelo-Arila C. Kniest dysplasia: radiologic, histopathological, and scanning electronmicroscopic findings. *Am.J.Med.Genet.* 1996;63:34-45.

106. Maumenee IH, Traboulsi EI. The Ocular Findings in Kniest Dysplasia. *Am.J.Ophthalmol.* 1985;100:155-60.

107. Rimoin DL, Siggers DC, Lachman RS, Silberberg R. Metatropic Dwarfism, Kniest Syndrome and Pseudoachondroplastic Dysplasias. *Clin.Orthop.Relat.Res.* 1976;70-82.

108. Rimoin DL, Hollister DW, Lachman RS, Kaufman RL, McAlister WH, Rosenthal RE et al. Histologic studies in the chondrodystrophies. *Birth Defects Orig.Artic.Ser.* 1974;10:274-95.

109. Wilkin DJ, Artz AS, South S, Lachman RS, Rimoin DL, Wilcox WR et al. Small deletions in the type II collagen triple helix produce Kniest dysplasia. *Am.J.Med. Genet.* 1999;85:105-12.

110. Marfan MAB. Un cas de deformation congenitale des quatre membres, plus prononcee aux extremites, caracterisee par l'allongement des os avec un certain degre d'amincissement. *Bull.Mem.Soc.Med.Hop.Paris* 1896;13:220-6.

111. Pyeritz RE, Murphy EA, McKusick VA. Clinical variability in the Marfan syndrome(s). *Birth Defects Orig.Artic.Ser.* 1979;15:155-78.

112. Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. *N.Engl.J.Med.* 1979;300:772-7.

113. De Paepe A, Devereux RB, Dietz HC, Hennekam RCM, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. *Am.J.Med.Genet.* 1996;62:417-26.

114. Bronner A, Kosmann M, Kosmann P. Le syndrome de Marfan (a propos de 71 cas). [Marfan's syndrome (apropos of 71 cases)]. *Bull.Soc.Ophtalmol.Fr.* 1976;76:631-9.

115. Cross HE, Jensen AD. Ocular manifestations in the Marfan syndrome and homocystinuria. *Am.J.Ophthalmol.* 1973;75:405-20.

116. Evain B, Langlois M, Francois P. Decollement de retine et syndrome de Marfan. [Retinal detachment and Marfan's syndrome]. *Bull.Soc.Ophtalmol.Fr.* 1986;86:875-82.

117. Farnsworth PN, Burke P, Dotto ME, Cinotti AA. Ultrastructural abnormalities in a Marfan's syndrome lens. *Arch.Ophthalmol.* 1977;95:1601-6.

118. Maumenee IH. The eye in the Marfan syndrome. *Trans.Am.Ophthalmol.Soc.* 1981;79:684-733.

119. Greco GM, Ambrosino L. Treatment of retinal detachment in Marfan syndrome. *Ann. Ophthalmol.* 1993;25:72-6.

120. Jarrett-WH II. Dislocation of the lens. A study of 166 hospitalized cases. *Arch. Ophthalmol.* 1967;78:289-96.

121. Loewenstein A, Barequet IS, De Juan E Jr, Maumenee IH. Retinal detachment in Marfan syndrome. *Retina* 2000;20:358-63.

122. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM et al. Marfan-Syndrome Caused by A Recurrent Denovo Missense Mutation in the Fibrillin Gene. *Nature* 1991;352:337-9.

123. Tynan K, Comeau K, Pearson M, Wilgenbus P, Levitt D, Gasner C et al. Mutation Screening of Complete Fibrillin-1 Coding Sequence - Report of 5 New Mutations, Including 2 in 8-Cysteine Domains. *Hum.Mol.Genet.* 1993;2:1813-21.

124. Dietz HC, Mcintosh I, Sakai LY, Corson GM, Chalberg SC, Pyeritz RE et al. Four Novel FBN1 Mutations - Significance for Mutant Transcript Level and EGF-Like Domain Calcium-Binding in the Pathogenesis of Marfan-Syndrome. *Genomics* 1993;17:468-75.

125. Knobloch WH, Layer IM. Retinal detachment and encephalocele. *J.Pediatr.Ophthal.* 1971;8:181-4.

126. Czeizel AE, Goblyos P, Kustos G, Mester E, Paraicz E. The Second Report of Knobloch Syndrome. *Am.J.Med.Genet.* 1992;42:777-9.

127. Seaver LH, Joffe L, Spark RP, Smith BL, Hoyme HE. Congenital Scalp Defects and Vitreoretinal Degeneration - Redefining the Knobloch Syndrome. *Am.J.Med.Genet.* 1993;46:203-8.

128. Passos-Bueno MR, Marie SK, Monteiro M, Neustein I, Whittle MR, Vainzof M et al. Knobloch Syndrome in A Large Brazilian Consanguineous Family - Confirmation of Autosomal Recessive Inheritance. *Am.J.Med.Genet.* 1994;52:170-3.

129. Wilson C, Aftimos S, Pereira A, Mckay R. Brief clinical report - Report of two sibs with Knobloch syndrome (encephalocoele and viteroretinal degeneration) and other anomalies. *Am.J.Med.Genet.* 1998;78:286-90.

130. Cook GR, Knobloch WH. Autosomal recessive vitreoretinopathy and encephaloceles. *Am.J.Ophthalmol.* 1982;94:18-25.

131. Suzuki OT, Sertie AL, Der Kaloustian VM, Kok F, Carpenter M, Murray J et al. Molecular analysis of collagen XVIII reveals novel mutations, presence of a third isoform, and possible genetic heterogeneity in Knobloch syndrome. *Am.J.Hum. Genet.* 2002;71:1320-9.

132. Menzel O, Bekkeheien RC, Reymond A, Fukai N, Boye E, Kosztolanyi G et al. Knobloch syndrome: Novel mutations in COL18A1, evidence for genetic heterogeneity, and a functionally impaired polymorphism in endostatin. *Hum.Mutat.* 2004;23:77-84.

133. Berger W, Ropers HH. In: Scriver CR, ed. *The Metabolic and Molecular Bases of Inherited Disease*. 8 ed. The McGraw-Hill Companies, Inc., 2001: 5977-85.

134. Welch RB, Goldberg MF. Sickle-Cell Hemoglobin and Its Relation to Fundus Abnormality. *Arch.Ophthalmol.* 1966;75:353-62.

135. Pemberton JW, Freeman HM, Schepens CL. Familial retinal detachment and the Ehlers-Danlos syndrome. *Arch.Ophthalmol.* 1966;76:817-24.

Chapter 2

Genetic risk of rhegmatogenous retinal detachment: a familial aggregation study

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2.1 Abstract

Objective:
To investigate the magnitude of the genetic risk of nonsyndromic rhegmatogenous retinal detachments (RRD) in a familial aggregation study.

Design:
Two hundred three consecutive patients with RRD and 461 controls without RRD were ascertained at the Department of Ophthalmology of the University Medical Centre Nijmegen in Nijmegen, the Netherlands. Data on family composition, history of RRD, and presence of other risk factors in siblings and offspring were collected by means of a questionnaire. Diagnosis of RRD was confirmed by evaluation of medical records.

Results:
One hundred eighty-one patients (89.2% of eligibles) and 408 controls (88.5% of invited controls) with 1090 and 2345 relatives, respectively, were included in the analysis. Thirteen familial RRDs (1.2%) were diagnosed in 10 case probands and 9 RRDs (0.4%) in 8 control probands. Siblings and offspring of cases had a higher incidence of RRD independent of age, sex, and myopia. The cumulative lifetime risk of RRD was 7.7% for relatives of cases and 3.0% for relatives of controls, yielding a risk ratio of 2.6 (95% confidence interval, 1.1-6.2).

Conclusions:
Familial occurrence of RRD is a risk factor for RRD. Genetic factors apart from myopia may explain the increased familial risk.

2.2 Introduction

Rhegmatogenous retinal detachment (RRD) is a common retinal disorder with an incidence of 1 per 10,000 individuals per year in Caucasians.[1-3] Untreated, RRD can lead to irreversible damage of the rods and cones, frequently ending in blindness.[4] Conversely, surgical treatment can achieve reattachment of the retina in 90 to 96% of cases when performed in a timely manner.[5-8] Prevention of RRD, however, is preferable. Preventive laser treatment and encirclement can be used in individuals with a known high risk of RRD.

Known endogenous risk factors for RRD are myopia, lattice degeneration and posterior vitreous detachment, while acquired risk factors such as trauma and prior cataract surgery can also contribute considerably to the risk of RRD. Furthermore, RRDs are more often found in men (57-60%) than in women[9;10], and race has proven to be an important determinant. A recent study showed that Chinese had an annual incidence of 11.6 per 100,000 individuals, Malays had an incidence of 7.0 per 100,000, and Indians had an incidence 3.9 per 100,000.[11] Blacks had an extremely low incidence; in black South-Africans, the incidence was reported to be as low as 0.46 per 100,000 individuals.[12] These racial differences may result from genetic disparity.

There is now ample evidence that retinal disorders related to RRD carry a genetic component. Case reports and twin studies have shown that retinal dialysis, idiopathic giant tears, lattice degeneration, and myopia can aggregate in families or can show a remarkable resemblance among relatives.[13-18] The genetic risk for myopia has been studied most profoundly, and a fifth genetic locus for high myopia was found recently.[19] Multiple RRDs, both with and without myopia, have been described in families. Some of these revealed inherited syndromic etiologies such as Wagner disease[20], erosive vitreoretinopathy[21] or Stickler syndrome[22], but others had no known syndromic cause of RRD.[23-25] Recently, we found evidence for genetic linkage in two large autosomal dominant RRD families without systemic abnormalities.[26]

Although genetic susceptibility to RRD appears to be present in specific families, it is currently unknown whether RRD occurring in a general population has a genetic etiology. The purpose of this study was to investigate the magnitude of the genetic risk of common, nonsyndromic RRD. We studied aggregation of RRD in siblings and offspring of probands with RRD that was not associated with other disorders, in comparison with siblings and offspring of healthy controls. In addition, we estimated the risk for first degree relatives independent of other risk factors.

2.3 Methods

The initial phase of the study, the selection of probands, had a case-control design. This was subsequently followed by construction of two cohorts of first-degree relatives for analysis.

Collection of probands
Probands were ascertained at the department of Ophthalmology of the University Medical Centre Nijmegen in Nijmegen, the Netherlands. The case group was composed of all consecutive patients with RRD who were hospitalized from June to December 2000. Exclusion criteria were non-Caucasian race; inherited retinal detachment-causing syndromes like Marfan, Wagner, Stickler and juvenile retinoschisis; and RRDs occurring less than 4 years after intra-ocular operations or ocular traumata.
The control group was composed of Caucasian companions of patients who were visiting our department for reasons other than RRD, who were not related to the patient-group, and who were matched within a five-year age range with case probands. Exclusion criteria for control probands were history of retinal detachment or (family) history of retinal detachment-causing syndromes, clinical symptoms of retinal detachment at the time of inclusion, and participation of relatives in the case group. Diagnoses of ophthalmologic disorders among the control-group were checked by medical records where appropriate. The study was executed according to the guidelines of the Committee on Research Involving Human Subjects Region Arnhem-Nijmegen, and written consent was obtained from all participants.

Data collection and diagnosis of RRD in relatives
Probands filled out a questionnaire regarding personal medical history, present symptoms of retinal detachment, and risk factors for RRD. The spherical equivalents of glasses or contact lenses were measured or collected from ophthalmologic records to obtain information about the presence and degree of myopia. The pre-operative refractive errors were used to assess myopia in subjects who had undergone cataract surgery. Myopia was defined as a spherical equivalent of -1,00 D or less in at least one eye.
Size, composition and ophthalmologic history of siblings and offspring of the proband were ascertained by questionnaire and subsequent personal follow-up by telephone by one of the investigators (SLG). Probands were asked for the ophthalmic history of each relative (e.g., a diagnosis of RRD, name of treating ophthalmologist, cataract extraction preceding RRD, trauma or other ophthalmologic events, and presence of myopia, as well as age at last contact). After informed consent and written permission of the affected relatives or their legal representatives was obtained, medical files of the ophthalmologists were collected in all subjects with histories of RRD and possible RRD-related disorders. A final diagnosis of RRD in relatives was given after

evaluation of these records by two independent retinal specialists (CBH and CCWK). Relatives of probands were excluded if they had never had contact with the proband, if their retinal detachment was due to a perforating trauma, or if the RRD was non-rhegmatogenous in origin.

Statistical analysis

Demographic and clinical characteristics were compared with t test for continuous variables and with $\chi 2$ test for categorical variables. Cox proportional hazards regression analysis was used to estimate the risk of RRD for first-degree relatives of cases independent of the confounding factors age, sex, and myopia. First-degree relatives of controls served as the reference. Each relative with RRD was tallied as a single event. The cumulative risk estimating the lifetime absolute risk of RRD for first-degree relatives was calculated with Cox proportional hazards analysis at age 85 years. Study participants 85 years or older were pooled to avoid biased estimates.[27]

2.4 Results

Family description

Of all consecutive 271 RRD cases that had been treated in the study period, a total of 68 cases were excluded because of inherited retinal detachment-causing syndromes (1 case), intra-ocular surgery within 4 years before the detachment (54 cases) or trauma (13 cases). The proband group that was eligible for our study consisted of 203 cases and 461 controls. Of these, 181 cases (89.2%) and 408 controls (88.5%) agreed to participate. The numbers of first-degree relatives generated by these probands were 1095 and 2346, respectively. Six subjects were excluded because they matched the exclusion criteria. In total, 1090 case relatives and 2345 control relatives entered the analysis.

Tables 2.1 and **2.2** present the distribution of demographic and clinical characteristics among probands and their relatives. Controls probands were, on average, 2.6 years younger and were more often female, while case probands more often had myopia and had undergone cataract extraction at a higher frequency. First-degree relatives were similar in most characteristics except the following: the mean age was significantly lower in relatives of controls, and myopia was significantly more often reported in relatives of cases.

Table 2.1 General characteristics of probands

		Case probands (n=181)	Control probands (n=408)	*P**
Age	Mean (SD)	58.6 (15.3)	56.0 (14.0)	0.045
Age group	<50 yrs (%)	40 (22)	142 (35)	
	50-70 yrs (%)	103 (57)	190 (47)	
	>70 yrs (%)	38 (21)	76 (19)	
Sex	Male (%)	118 (65)	172 (42)	<0.001
	Female (%)	63 (35)	236 (58)	
Family size	Mean (SD)	6.0 (3.6)	5.8 (3.4)	0.31
No. of siblings	Mean (SD)	4.1 (2.9)	3.9 (2.7)	0.51
No. of offspring	Mean (SD)	2.0 (1.7)	1.9 (1.4)	0.90
Myopia	Number (%)	116 (64)	136 (13)	<0.001
Surgery	Lensectomy (%)	43 (24)	20 (4.9)	<0.001
	Vitrectomy (%)	2 (1.2)	1 (0.2)	0.18
	Glaucoma (%)	0	2 (0.5)	0.61
	Strabismus (%)	3 (1.7)	4 (1.0)	0.23

* adjusted for age and sex where appropriate

Table 2.2 General characteristics of first degree relatives*

	Siblings			Offspring		
	Cases n=736	**Controls n=1572**	**P**	**Cases n=354**	**Controls n=773**	**P**
Mean age in yrs (SD)	57.95 (15.9)†	55.36 (15.8)	<0.001	33.23 (12.3)†	28.75 (13.5)	<0.001
Age group						
<50 yrs	183 (24.9)†	540 (34.4)		333 (94.1)	742 (95.9)	
50-70 yrs	395 (53.7)‡	753 (47.9)		21 (5.9)	29 (3.8)	
>70 yrs	158 (21.5)‡	279 (17.7)		0 (0.0)	2 (0.3)	
Sex						
Male	349 (47.4)	801 (51.0)	0.11	176 (49.7)	406 (52.5)	0.38
Female	387 (52.6)	771 (49.0)		178 (50.3)	367 (47.5)	
Ocular pathology						
Myopia	187 (25.4)‡	337 (21.4)	0.05	111 (31.4)†	162 (21.0)	0.003
Glaucoma	9 (1.2)	15 (1.0)	0.56	0 (0.0)	2 (0.3)	0.3
Macular degeneration	3 (0.4)	3 (0.2)	0.48	0 (0.0)	0 (0.0)	

* *P* adjusted for age and sex when appropriate; % between brackets unless described otherwise; † *P* <0.001, ‡ *P* <0.05

Risk of RRD for first degree relatives

Retinal detachments in relatives were initially reported by 13 case probands and 15 control probands. Validation of the diagnosis of RRD by medical reports reduced the numbers to 13 relatives of 10 case probands and 9 relatives of 8 control probands. Misclassification of the reported RRD was caused by confusion with a large range of other ophthalmic pathology, varying from cataract extraction to removal of epiretinal membranes.

The relative group that showed a significant higher absolute RRD frequency between cases and controls was the sibling group (n=12 [1.6%] versus n=7 [0.4%]; *P*=0.01); there was no statistically significant difference in absolute RRD frequency in offspring. A summary of the number of all affected first-degree relatives per proband is given in **Table 2.3**. **Table 2.4** presents the frequency distribution and the risk of RRD for first-degree relatives. The annual incidence of RRD in relatives of cases was estimated at 23.9 per 100,000 individuals, and in relatives of controls 8.24 per 100,000.

Table 2.3 Frequencies of rhegmatogenous retinal detachment in siblings and offspring per proband

Total number of affected	Case probands (n=181)			Control probands (n=408)		
	No. (%)	No. with affected siblings	No. with affected offspring	No. (%)	No. with affected siblings	No. with affected offspring
0	171 (94.4)	0	0	400 (98.1)	0	0
1	8 (4.4)	7	1	7 (1.7)	5	2
2	1 (0.6)	1	0	1 (0.2)	1	0
3	1 (0.6)	1	0	0	0	0

Table 2.4 Risk of rhegmatogenous retinal detachment (RRD) for first degree relatives

	Total	No. RRD absent	No. RRD present				Risk of RRD (95%CI)			
			After trauma, (%)	<4 yrs after cataract extraction, (%)	≥4 yrs after cataract extraction, (%)	Spontaneous, (%)	All types		Spontaneous	
							Crude relative risk	Adjusted relative risk†	Crude relative risk	Adjusted relative risk†
Siblings of										
cases	736	724	1 (0.14)	1 (0.14)	1 (0.14)	9 (1.2)	3.1 (1.2-7.9)	3.0 (1.2-7.5)	3.3 (1.1-9.7)	3.1 (1.0-9.3)
controls	1572	1565	1 (0.06)	1 (0.06)	0	5 (0.3)				
Offspring of										
cases	354	353	0	0	0	1 (0.3)	*	*	*	*
controls	773	771	0	0	0	2 (0.3)				

* Not enough events for analysis; † Adjusted for age, sex, and myopia

Bilaterality of RRD was present in one (8%) of 13 affected relatives of cases. The interval between the RRDs was 2 years. In controls, none of the nine affected relatives had a bilateral RRD. Retinal breaks without retinal detachment in fellow eyes were found in another two relatives of cases (15.4%), and in only one relative of controls (11.1%). These breaks were round atrophic holes in both case relatives, and a cribrous area in the control relative. All were seen within 2 years of the event. One patient from the case group had undergone laser treatment.

Figure 2.1 shows the cumulative lifetime risk of all types of RRD as a function of age for the total group of siblings and offspring. Relatives of cases had a higher frequency of RRD. Beyond 50 years, an increasing discrepancy was found between the two groups. Not only did the risk of RRD appear higher for relatives of cases, the curve had also shifted to the left, implying an earlier onset of RRD among case families. However, before the age of 69 years there was no statistically significant difference. The lifetime absolute risk at age 85 years for case relatives was 7.7% and for control relatives, 3.0%. This resulted in a risk ratio of 2.6 (95% confidence interval, 1.1-6.2).

Case and control probands with myopia had more affected relatives than probands without myopia. Of all 1333 relatives derived from case and control probands with myopia, 1.13% were diagnosed with RRD versus 0.33% of 2102 relatives of probands without myopia. This resulted in an age- and sex-adjusted odds ratio of 4.04 (95% confidence interval, 1.64-9.96). The frequency difference of relatives with RRD

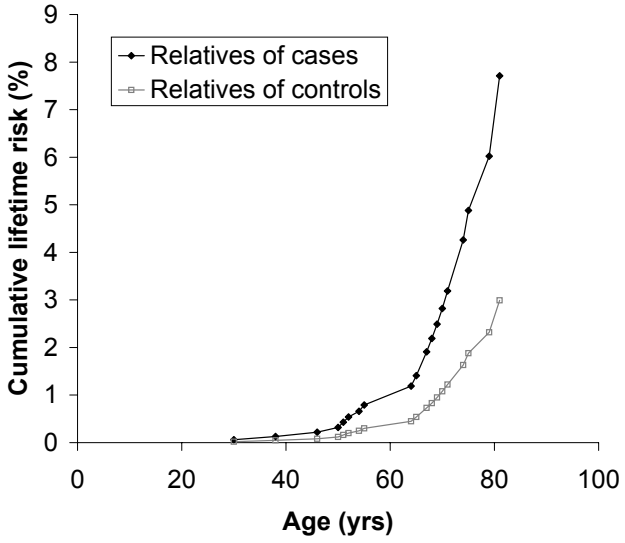

Figure 2.1 Cumulative lifetime risk of all types of rhegmatogenous retinal detachment as a function of age for siblings and offspring of cases versus siblings and offspring of controls

between probands with and without myopia was more prominent in the case group: 1.79% versus 0.46% (P=0.04) in the case group and 0.56% versus 0.31% (P=0.47) in the control group. Moreover, there was a significantly higher frequency of RRD in relatives of case probands with myopia versus control probands with myopia (1.8% vs. 0.6%); P=0.03, suggesting interaction between myopia and proband status. However, the interaction term (myopia * proband status) did not reach statistical significance when added to the model in **Table 2.4** (P=0.60).

2.5 Comment

Our data show the presence of familial predisposition to nonsyndromic RRD. Siblings of subjects with this type of RRD had a threefold increased frequency of RRD compared with siblings of nonaffected subjects. This familial risk was not fully explained by the aggregation of known risk factors myopia, age and sex.

Our study was designed to compare the risks for RRD between affected relatives of unselected, consecutive RRD patients and healthy controls derived from the same district in the Netherlands. We limited our family data collection to siblings and offspring to enhance the reliability of clinical history. The data collection was performed in an identical fashion among both study groups. All histories of RRD were verified using medical records and the general diagnostic criteria of good clinical practice were applied. For probands we used stringent criteria and accepted only spontaneous nonsyndromic RRD, whereas for relatives we registered all types of RRD to examine the entire disease spectrum.

A limitation in our study is the low frequency of the disorder. We investigated a large population of relatives, but the number of outcome events was still small among both study groups. This limited the power of the analyses, although we were able to detect significant differences between groups. Another potential drawback was the reliance on family history for ascertainment of RRD. We do not think that this has distorted our results, because RRD appears to be a significant ophthalmologic event that has a great and memorable impact on patients and their families. In addition, the intensive validation procedure by medical records and adjudication by retinal specialists facilitated the diagnosis of genuine nonsyndromic RRD. Frequencies of reported RRD among the relatives were comparable with former reports.[28;29] The annual incidence of RRD of 8.24 per 100,000 individuals in our control group was comparable with the annual incidences mentioned in British epidemiologic studies: 6.3 to 13.0 per 100,000 subjects.[30] Bilaterality of familial RRD appeared to be lower than the 15% to 25% in other family reports.[28;31] The low frequency of retinal breaks and treatment in the second eye suggested that this could not be explained by higher awareness and preventative treatment. These other reports possibly harbour more high-risk families.

Myopia was an important risk factor for RRD in our study. Although a negative refractive error appeared to enhance the risk of RRD among relatives of both proband groups, the risk was higher for families of case probands. Former reports regarding familial nonsyndromic RRD predominantly describe myopic families.[18;23;24;28;32] The genetic background of myopia is still unclear, but linkage in specific families has been found with five different loci (Xq28; 18p11.31; 12q21-q23; 7q36; 17q21-q22).[19;33-36] Cataract extractions did not precede RRD considerably more frequent in relatives of cases than in relatives of controls, indicating that cataract extraction did not explain familial aggregation of RRD in our study.

Our data suggest that genetic risk factors for RRD, other than those determining axial

length, exist, because myopia did not fully explain the increased risk in first-degree relatives in our study. Moreover, our data implying interaction between myopia and proband status may indicate that myopia genes have influence on these other genetic determinants. Additional support for the existence of genes not involving myopia comes from former family reports that describe autosomal dominant occurrence of spontaneous nonsyndromic RRD without myopia.[24-26] In a previous report, we presented evidence for linkage with the region containing the *COL2A1* gene in two unrelated RRD families, and identification of the pathogenic mutation in one family. *COL2A1* codes for the three α1-chains of collagen type II and for the precursor of the α3-chain of collagen type XI, a collagen that forms the core of collagen type II.[37;38] This collagen is important for the structure of the vitreous and maintenance of the vitreoretinal interface, and it is likely that dysfunction increases the risk of RRD. Other genes serving similar functions may exist.

Do RRD families require specific clinical care? The current policy for treatment is described in the American Academy of Ophthalmology Preferred Practice Pattern.[39] It does not offer specific guidance in clinical management of relatives of patients with RRD. Our demonstration of familial aggregation of RRD suggests that these patients require extra awareness of prodromal symptoms. Further investigation is needed to decide if preventative treatment for retinal detachment should be performed at an earlier stage in these relatives, especially in those with myopia.

2.6 Acknowledgments

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References

1. Wilkes SR, Beard CM, Kurland LT, Robertson DM, O'Fallon WM. The incidence of retinal detachment in Rochester, Minnesota, 1970-1978. *Am.J.Ophthalmol.* 1982;94:670-3.
2. Schepens CL, Marden D. Data on the Natural History of Retinal Detachment: I. Age and Sex Relationships. *Arch.Ophthalmol.* 1961;66:631-42.
3. Sasaki K, Ideta H, Yonemoto J, Tanaka S, Hirose A, Oka C. Epidemiologic characteristics of rhegmatogenous retinal detachment in Kumamoto, Japan. *Graefes Arch.Clin.Exp. Ophthalmol.* 1995;233:772-6.
4. Ivanisevic M. The natural history of untreated rhegmatogenous retinal detachment. *Ophthalmologica* 1997;211:90-2.
5. The repair of rhegmatogenous retinal detachments. American Academy of Ophthalmology. *Ophthalmology* 1990;97:1562-72.
6. Ross WH, Kozy DW. Visual recovery in macula-off rhegmatogenous retinal detachments. *Ophthalmology* 1998;105:2149-53.
7. Ah-Fat FG, Sharma MC, Majid MA, McGalliard JN, Wong D. Trends in vitreoretinal surgery at a tertiary referral centre: 1987 to 1996. *Br.J.Ophthalmol.* 1999;83:396-8.
8. Minihan M, Tanner V, Williamson TH. Primary rhegmatogenous retinal detachment: 20 years of change. *Br.J.Ophthalmol.* 2001;85:546-8.
9. Algvere PV, Jahnberg P, Textorius O. The Swedish Retinal Detachment Register. I. A database for epidemiological and clinical studies. *Graefes Arch.Clin.Exp.Ophthalmol.* 1999;237:137-44.
10. Ivanisevic M, Bojic L, Eterovic D. Epidemiological study of nontraumatic phakic rhegmatogenous retinal detachment. *Ophthalmic Res.* 2000;32:237-9.
11. Wong TY, Tielsch JM, Schein OD. Racial difference in the incidence of retinal detachment in Singapore. *Arch.Ophthalmol.* 1999;117:379-83.
12. Peters AL. Retinal Detachment in Black South Africans. *S.Afr.Med.J.* 1995;85:158-9.
13. Teikari JM, Odonnell J, Kaprio J, Koskenvuo M. Impact of Heredity in Myopia. *Hum. Hered.* 1991;41:151-6.
14. Verdaguer TJ, Rojas B, Lechuga M. Genetical studies in nontraumatic retinal dialysis. *Mod.Probl.Ophthalmol.* 1975;15:34-9.
15. Vaiser A, Jost BF. Bilateral Inferotemporal Dialysis in Identical Twins. *Ann.Ophthalmol.* 1992;24:378-80.
16. Chaudhry NA, Flynn HW, Jr., Tabandeh H. Idiopathic giant retinal tears in identical twins. *Am.J.Ophthalmol.* 1999;127:96-9.
17. Chen CJ, Cohen BH, Diamond EL. Genetic and Environmental Effects on the Development of Myopia in Chinese Twin Children. *Ophthalmic Paediatr.Genet.* 1985;6:113-9.

18. Lewkonia I, Davies MS, Salmon JD. Lattice Degeneration in A Family: with Retinal Detachment and Cataract. *Br.J.Ophthalmol.* 1973;57:566-71.

19. Paluru P, Ronan SM, Heon E, Devoto M, Wildenberg SC, Scavello G et al. New locus for autosomal dominant high myopia maps to the long arm of chromosome 17. *Invest. Ophthalmol.Vis.Sci.* 2003;44:1830-6.

20. Graemiger RA, Niemeyer G, Schneeberger SA, Messmer EP. Wagner vitreoretinal degeneration. Follow-up of the original pedigree. *Ophthalmology* 1995;102:1830-9.

21. Brown DM, Kimura AE, Weingeist TA, Stone EM. Erosive vitreoretinopathy. A new clinical entity. *Ophthalmology* 1994;101:694-704.

22. Stickler GB. Hereditary progressive arthro-ophthalmopathy. *Mayo Clin.Proc.* 1965;40:433-55.

23. Gornig H. [Familial retinal detachment]. *Dtsch.Gesundheitsw.* 1966;21:1662-5.

24. Haut J, Limon S, Offret H, Morax S. [Familial peripheral retinal degenerescence and retinal detachment]. *Bull.Soc.Ophtalmol.Fr.* 1973;73:27-37.

25. Saari KM. Autosomal dominant juvenile vitreoretinal degeneration and retinal detachment. *Int.Ophthalmol.* 1986;9:45-60.

26. Go SL, Maugeri A, Mulder JJS, van Driel MA, Cremers FPM, Hoyng CB. Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the COL2A1 gene. *Invest.Ophthalmol.Vis.Sci.* 2003;44:4035-43.

27. Chase GA, Folstein MF, Breitner JCS, Beaty TH, Self SG. The Use of Life Tables and Survival Analysis in Testing Genetic Hypotheses, with An Application to Alzheimer‘s Disease. *Am.J.Epidemiol.* 1983;117:590-7.

28. Cuendet JF, Gailloud C, Dufour R. [Genetics and idiopathic retinal detachment]. *Mod. Probl.Ophthalmol.* 1975;15:10-21.

29. Cuendet JF. Genetique. *Bibl.Ophthalmol.* 1965;46-54.

30. Mowatt L, Shun-Shin G, Price N. Ethnic differences in the demand incidence of retinal detachments in two districts in the West Midlands. *Eye* 2003;17:63-70.

31. Scherman O, Tornquist R. Inherited Retinal Detachment. *Acta Ophthalmol.(Copenh)* 1981;59:642-8.

32. Thomas C, Cordier J, Saudax E, Raspiller A. A propos du decollement familial de la retine. [Apropos of the familial retinal detachment]. *Bull.Soc.Ophtalmol.Fr.* 1967;67:1166-70.

33. Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS et al. Evidence that a locus for familial high myopia maps to chromosome 18p. *Am.J.Hum. Genet.* 1998;63:109-19.

34. Young TL, Ronan SM, Alvear AB, Wildenberg SC, Oetting WS, Atwood LD et al. A second locus for familial high myopia maps to chromosome 12q. *Am.J.Hum.Genet.* 1998;63:1419-24.

35. Naiglin L, Gazagne C, Dallongeville F, Thalamas C, Idder A, Rascol O et al. A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. *J.Med. Genet.* 2002;39:118-24.

36. Schwartz M, Haim M, Skarsholm D. X-linked myopia: Bornholm eye disease. Linkage to DNA markers on the distal part of Xq. *Clin.Genet.* 1990;38:281-6.

37. Brown JC, Timpl R. The collagen superfamily. *Int.Arch.Allergy Immunol.* 1995;107:484-90.

38. Myllyharju J, Kivirikko KI. Collagens and collagen-related diseases. *Ann.Med.* 2001;33:7-21.

39. American Academy of Ophthalmology. Preferred Practice Pattern: Posterior Vitreous Detachment, Retinal Breaks, and Lattice Degeneration. Available from http://www.aao.org/aao/education/library/ppp. 2003.

Chapter 3

Autosomal dominant rhegmatogenous retinal detachment – clinical appearance and surgical outcome

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3.1 Abstract

Purpose:
To study the clinical appearance and surgical results of autosomal dominantly inherited, rhegmatogenous retinal detachments (RRDs).

Methods:
After prospective examination of all but two family members, the medical records of 16 affected patients (21 eyes) of two families from the Netherlands with autosomal dominantly inherited RRD were retrospectively evaluated. Special attention was paid to the age at onset, the ocular morphology and the clinical appearance of the RRD. The type and number of the various surgical procedures were analyzed with respect to preoperative appearance of the RRD, postoperative results and final visual acuity.

Results:
The mean age at onset of RRD of affected individuals in family A and B was 37±18 years and 19±10 years, respectively. The mean ocular axial length in the two families was 24.7 mm and 26.7 mm. The mean number of retinal defects preoperatively found was 2.2 in family A and 7.1 in family B. Round, atrophic retinal holes predominated. Two of 21 affected eyes showed significant preoperative proliferative vitreoretinopathy. Pars plana vitrectomy was the primary procedure in four cases; extra ocular buckling was the initial procedure in 15 cases. One eye received scleral folding with diathermy as primary surgery. Redetachment following surgery occurred in 5 of 10 cases in family A and 4 of 10 eyes in family B. Anatomical success could be achieved in 9 of 10 and 8 of 10 eyes in family A and B, respectively.

Conclusions:
In these families the prevalence of RRD is high. Most patients were affected at a relatively young age compared with non-genetically linked forms of RRD. Because of the low success rate of surgical intervention and, subsequently, the high number of operations necessary to achieve reattachment of the retina, the use of diagnostic genetic techniques to identify individuals at risk would be advisable. In these subjects measures to prevent RRD are an option, even when anatomical substrates of precursors of RRD are absent.

3.2 Introduction

Rhegmatogenous retinal detachment (RRD) is a potential disastrous ocular disease which is defined as a separation of the neurosensory retina from the retinal pigment epithelium caused by a retinal break. The incidence of RRD is about 10 per 100,000 population per year.[1;2] If left untreated, the majority of cases of RRD will progress to complete detachment and result in blindness. Surgical intervention results in primary reattachment of the retina in about 76-85% of the cases. Overall, the success rate of surgery, defined in anatomical and not functional terms, is approximately 90-95%.[3;4]
The clinical appearance of RRD of presumed autosomal dominant origin has been reported.[5;6] However, most of the patients described demonstrate additional ocular or systemic abnormalities, suggesting an underlying syndrome. An association with RRD is well known in autosomal dominant inherited disorders such as Stickler and Jansen syndrome, as well as in Wagner disease.[7;8] In contrast to Wagner disease, the frequency of RRD in Jansen and in Stickler syndrome is significantly higher. In all of these syndromic disorders specific ocular and systemic abnormalities form typical phenotypes.[8-12] Moreover, a number of connective tissue disorders associated with vitreoretinal degenerations and RRD have been reported, e.g., Kniest dysplasia, osteogenesis imperfecta, spondyloepiphyseal dysplasia congenita and Ehlers-Danlos syndrome.[13-16]
Surgery of RRD associated with syndromic ocular disorders such as Stickler syndrome and Wagner disease is frequently disappointing, and recurrence of RRD is common.[7;11] However, there is little information about the results of surgery in nonsyndromic forms of inherited RRD.
In this study we report the clinical characteristics and surgical outcome of affected members of two families with autosomal dominantly inherited RRD. In neither family were the classic ocular and systemic features of complex hereditary vitreoretinopathies such as Wagner disease and Stickler syndrome recorded, as described elsewhere.[17]
Our study was set out to establish whether the surgical outcome of patients suffering from nonsyndromic autosomal dominant RRD is as poor as the results of surgery in syndromic RRD reported in the literature.

3.3 Patients and Methods

Our study was designed as a cross-sectional study, including (a) a prospective clinical investigation of affected and unaffected members of two families with autosomal dominantly inherited RRD and (b) a retrospective study of the clinical appearance and surgical results of RRD in affected family members.

We studied two unrelated families from the Netherlands (families A and B) with autosomal dominantly inherited RRD. DNA linkage analysis in all family members had shown that the RRD phenotype in both families cosegregated with the 12q13 region containing the *COL2A1* gene. In family A no mutation could be identified in the protein coding exons of *COL2A1*; in family B, an Arg435Ter mutation located in the triple helical domain of the *COL2A1* gene was found.[17]

The study was divided into two parts. The first part comprised a prospective investigation including affected and nonaffected members of both families. All 29 members of family A and in 40 members of family B were questioned in detail as to their medical history, focusing on ophthalmic, audiologic, cardiologic and orthopaedic aspects, to exclude the presence of an underlying syndrome. In two members of family B, such an interview was not possible. The eyes of all other subjects were prospectively examined by two experienced investigators (SLG and CBH) and underwent extensive inspection by binocular contact lens biomicroscopy and slit-lamp microscopy in order to exclude features of syndromic ocular diseases. We paid particular attention to cataract formation, vitreous degenerations and membranes, and specific retinal changes. If possible, the axial lengths of the eyes were recorded with applanation ultrasound biometry (Ocuscan; Alcon, Irvine, CA, USA). General physical examination was performed to detect possible abnormalities of the palate, face, joints and heart sounds. In affected individuals who were willing to cooperate, audiometry was carried out (6 members of family A and 10 members of family B).

In the second part of our study we retrospectively evaluated the medical records of the 16 affected individuals (21 eyes) of these two families. All patients with RRD had undergone vitreoretinal surgery in our department. The records of these patients were reviewed for preoperative, operative and postoperative data. Proliferative vitreoretinopathy (PVR) was classified according to the updated classification published in 1991 by the Retina Society.[18] Due to the long period of time over which these patients presented, a number of different vitreoretinal surgeons were involved and various surgical techniques were used. All surgeons involved were experienced and based their diagnoses and surgical decisions on up-to-date literature. Moreover, all surgeons were familiar with the features of the syndromic disorders mentioned above.

3.4 Results

The pedigrees of family A and B are depicted in **Figures 3.1** and **3.2**. Only 1 of 21 affected eyes showed a juvenile cataract (individual BII-10). However, none of the remaining family members showed classic ocular or systemic features associated with Wagner disease, Stickler syndrome or other connective tissue disorders that might be associated with RRD, as detailed elsewhere.[17] We found lattice degeneration in some family members, but not in any subject with RRD.
At the point of retrospective analysis the mean duration of follow-up of affected individuals was 7 years (range 1-9 years). The average age of onset of RRD in affected patients was 37 years (range 15-64 years) in family A and 19 years (range 6 to 35 years) in family B. RRD of the fellow eye occurred in 3 of 8 patients in family A and in 2 of 8 patients in family B.
The number of preoperatively localized retinal defects was 2.2 (range 0-7) in family A and 7.1 (range 1-28) in family B. Details of localization, type and size of retinal holes are listed in **Table 3.1**. Five cases in family A and two cases in family B had minimal preoperative PVR (grade A). In Family A, we found two eyes with serious PVR before first surgery. Further details concerning PVR are given in **Table 3.1**.

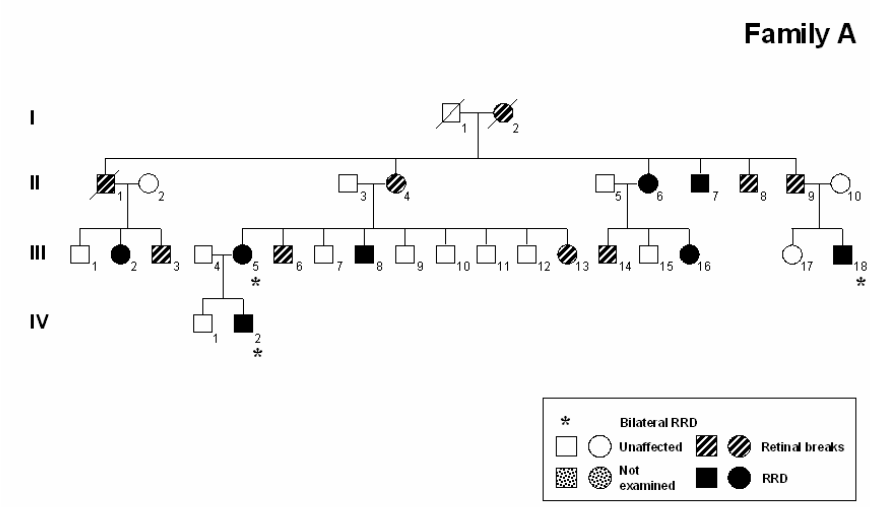

Figure 3.1 Pedigree of family A, showing an autosomal dominant mode of inheritance of rhegmatogenous retinal detachments

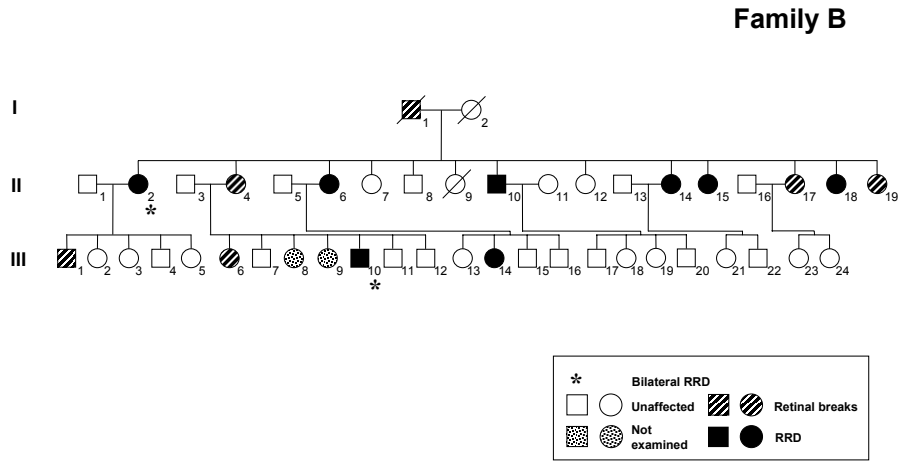

Figure 3.2 Pedigree of family B, showing an autosomal dominant mode of inheritance of rhegmatogenous retinal detachments

In both families, we observed moderate to high myopia with an average ocular axis length of 24.7 mm (range 20.2 mm to 28.2 mm) in family A and 26.7 mm (range 25.4 mm to 28.7 mm) in family B.
The mean number of operations was 2.4 (range 1-6) in family A and 2.5 (range 1-8) in family B. Eventually, the retina remained detached in two patients of family A and in one patient of family B. Surgical failure after the initial procedure was attributed to PVR in four of five cases in family A and in three of four cases in family B. Surgical data are listed in detail in **Table 3.1**. The average final visual acuity was 20/80 (range 20/20 to no light perception) in family A and 20/50 (range 20/25 to no light perception) in family B. In 3 of 10 treated eyes in family A and in 3 of 10 operated eyes in family B, final visual acuity was hand motions or worse. For further details see **Table 3.1**.

Table 3.1 Clinical and surgical data for the 21 eyes with inherited retinal detachment

								Surgical Procedure				Visual acuity				
Patient No.	**Eye No.**	**Sex/Eye**	**Age at Onset of RRD**	**Number of Defects/ Quadrants detached**	**Localization/ Type of defects**	**PVR Grade**	**Axial length (mm)**	**First Operation**	**Second Operation**	**Last Operation**	**Total Number of Operations**	**Initial**	**Final**	**Final retinal state**	**Lens/ vitreous abnormalities**	**Complications/ Remarks**
AII-6	A 1	F/OD	60	0/4	-	Cp 12	24.0	PPV, oil	SBP	-	2	LP	LP	A	Scat/-	-
AII-7	A 2	M/OS	64	?/4	-	Cp 12	20.2	-	-	-	0	NLP	NLP	D	Scat/-	Funnel
AIII-2	A 3	F/OD	35	-	-	-	-	SBP	-	-	1	-	20/200	A	Scat/-	-
AIII-5	A 4	F/OD	45	2/1	TS, TI/HS	A	-	PPV, SBP, gas	PPV, oil	PPV, oil	4	20/32	HM	A	-	-
AIII-5	A 5	F/OS	49	1/2	TI, NS/HS, RH	-	23.5	SBP	-	-	1	-	-	A	Scat/-	-
AIII-8	A 6	M/OD	29	7/3	TS, TI/HS, RH	A	28.2	SBP	-	-	1	HM	20/63	A	Cl/-	-
AIII-16	A 7	F/OS	33	2/3	NS/RH	A	24.7	SBP	-	-	1	20/25	20/20	A	Cl/-	-
AIII-18	A 8	M/OD	15	3/4	TS, TI/RH	0	25.7	SBP	-	-	1	CF	20/200	A	Cl/-	Secondary strabismus
AIII-18	A 9	M/OS	16	3/1	TS, TI/RH	0	26.7	SBP	SBP	-	2	20/20	20/20	A	Cl/-	-
AIV-2	A 10	M/OD	16	1/1	TS/GT	A	-	SBP	PPV, oil	SOR	5	20/25	NLP	D	Secat/-	Hypotony, keratopathy
AIV-2	A 11	M/OS	19	1/2	NS/ORA	A	-	PPV, SBP, gas	PPV, SBP	PPV, oil	6	20/25	CF	A	Secat/-	-

(RRD=rhegmatogenous retinal detachment; PVR=proliferative vitreoretinopathy; TS=temporal superior quadrant; TI=temporal inferior quadrant; NS=nasal superior quadrant; NI=nasal inferior quadrant; HS=horse shoe tear; RH=round retinal hole; ORA=oradialysis; GT=giant retinal tear; PPV=pars plana vitrectomy; SBP=scleral buckling procedure; gas=intraocular gas tamponade; oil=intraocular silicone oil tamponade; SOR=silicone oil removal; CF=counting fingers; HM=hand motions; LP=light perception; NLP=no light perception; A=attached; D=detached; cl=clear lens; Pf=pseudophakic; scat=senile cataract; secat=secondary cataract after vitrectomy) *(Table continued on next page)*

Patient No.	Eye No.	Sex/Eye	Age at Onset of RRD	Number of Defects/ Quadrants detached	Localization/ Type of defects	PVR Grade	Axial length (mm)	Surgical Procedure				Visual acuity		Final retinal state	Lens/ vitreous abnormalities	Complications/ Remarks
								First Operation	Second Operation	Last Operation	Total Number of Operations	Initial	Final			
BII-2	B 1	F/OD	11	8/3	TS,TI,NI/RH	-	26.1	Scleral excision + diathermy	SBP	PPV + SBP	3	CF	20/63	A	Cl/-	-
BII-2	B 2	F/OS	17	28/3	TS, TI, NI/RH	0	28.5	SBP	-	-	1	LP	NLP	A	Cl/-	Ocular ischemia
BII-6	B 3	F/OD	17	7/3	TS, TI, NI/HS, RH	0	-	SBP	PPV	PPV, oil	5	20/40	LP	D	Secat/-	-
BII-10	B 4	M/OS	35	2/3	NI/RH	0	26.1	PPV, SBP, gas	-	-	1	20/200	20/200	A	Pf/-	History of laser treatment for local retinal detachment (age 27 years); cataract extraction (age 33 years)
BII-14	B 5	F/OD	27	7/1	TS/RH	0	27.6	SBP	-	-	1	20/32	20/32	A	Cl/-	Additional laser treatment
BII-15	B 6	F/OS	22	11/2	TS, TI/RH	A	28.7	SBP	SBP	PPV, oil	8	20/200	HM	A	Secat/-	-
BII-18	B 7	F/OS	27	2/2	TS/RH	0	26.4	SBP	-	-	1	CF	20/50	A	Cl/-	-
BIII-10	B 8	M/OD	9	3/2	TS/RH	A	25.4	SBP	-	-	1	20/200	20/25	A	Cl/-	-
BIII-10	B 9	M/OS	9	1/3	NS/RH	0	25.7	SBP	-	-	1	20/200	20/25	A	Cl/-	-
BIII-14	B 10	F/OD	6	2/3	TS, TI/HS	0	25.8	SBP	Buckle removal + laser	SBP	3	CF	20/32	A	Cl/-	-

3.5 Discussion

Autosomal dominantly inherited vitreoretinal disorders frequently lead to RRD, and in such cases the outcome of surgery has been reported as poor.[7;15;16] To our knowledge, there have been no reports about surgery of inherited RRD unassociated with other ocular or systemic disorders.

In both of the families in our study, the underlying genetic defect was located in the chromosomal region containing the *COL2A1* gene, and in one of the families (family B), a protein-truncating mutation was found in the middle of the helical domain of the *COL2A1* gene, a type of mutation generally found in patients with classic Stickler syndrome.[17] To date, there is no clear explanation for this finding. In a family as large as family B it is remarkable that not a single patient showed the features of classic Stickler syndrome. Although an atypical ocular form of Stickler syndrome cannot be excluded, predominantly ocular Stickler syndromes have been associated with *COL2A1* exon 2 mutations.[19] In contrast, the genetic defect found in family B was an exon 30 mutation.[17]

At presentation, the majority of the patients reported in our study were relative young. Especially in family B the average age of 18 years was strikingly low. In contrast, the mean age of patients with sporadic RRD is about 60 years.[20;21] Interestingly, RRD in our patients was predominantly caused by a high number of round, atrophic holes rather than by characteristic horseshoe tears, which are typical for sporadic RRD. Our findings concerning retinal defects correlate with those observed in RRD patients with high myopia, Kniest dysplasia and Stickler syndrome.[7;15;22]

In our group of patients a marked number of bilateral RRDs occurred. Three of eight patients in family A and two of eight patients in family B, e.g. almost one third of all RRD patients, were bilaterally affected. This significantly increased risk for the fellow eye is not so obvious in sporadic cases, where bilaterality occurs in about 4,2-19% of those affected.[1;2;20;22]

Both the young age of our patients and the fact the RRDs seem to be caused by small atrophic holes rather than horseshoe tears suggest that an intrinsic fragility of the retina rather than traction at the vitreoretinal interface is responsible for this disorder. Although none of the affected subjects in our study demonstrated any symptoms that can be assumed to be part of a syndromic disease, the poor outcome of RRD surgery in our patients corresponds with the equally disappointing results in RRDs associated with Stickler syndrome.[7] This unfortunate surgical prognosis is in contradiction to the outcome of surgery in sporadic, nonsyndromic RRD.[3;4]

The poor surgical results in our patients were independent of the surgical technique used. Moreover, the surgical outcome in recently treated patients from our families was not better than the results in family members operated on more than 20 years ago. Thus, individuals suffering from autosomal dominantly inherited RRD may not benefit from the recent advances in vitreoretinal surgical techniques.

Due to the poor surgical success rates timely identification of subjects at risk is desirable in order to prevent the manifestation of RRD. Moreover, genetic evaluation could identify even very young individuals at risk.

Although asymptomatic retinal defects in phakic eyes did not significantly increase the risk for sporadic RRD in long-term observation[23], prophylactic treatment of defects has been advised for patients with Stickler syndrome and other vitreoretinal hereditary diseases.[24] Consequently, we propose prophylactic laser treatment of genetically affected family members with retinal defects, which should cover the entire 360° because of the speculated intrinsic fragility of the retinal periphery. However, retinopexy should be carried out very gently in view of the reported posterior vitreous detachment after prophylactic laser treatment in inherited RRD[7] and because of the high prevalence of PVR in our patients. To date, we have not been able to identify genetically affected subjects who were asymptomatic. Therefore, the benefit of prophylactic treatment in those family members is only speculative.

3.6 Acknowledgments

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References

1. Sasaki K, Ideta H, Yonemoto J, Tanaka S, Hirose A, Oka C. Epidemiologic characteristics of rhegmatogenous retinal detachment in Kumamoto, Japan. *Graefes Arch.Clin.Exp.Ophthalmol.* 1995;233:772-6.
2. Tornquist R, Stenkula S, Tornquist P. Retinal Detachment - A Study of A Population-Based Patient Material in Sweden 1971-1981 .1. Epidemiology. *Acta Ophthalmol.(Copenh).* 1987;65:213-22.
3. Ah-Fat FG, Sharma MC, Majid MA, McGalliard JN, Wong D. Trends in vitreoretinal surgery at a tertiary referral centre: 1987 to 1996. *Br.J.Ophthalmol.* 1999;83:396-8.
4. The repair of rhegmatogenous retinal detachments. American Academy of Ophthalmology. *Ophthalmology* 1990;97:1562-72.
5. Gornig H. [Familial retinal detachment]. *Dtsch.Gesundheitsw.* 1966;21:1662-5.
6. Saari KM. Autosomal dominant juvenile vitreoretinal degeneration and retinal detachment. *Int.Ophthalmol.* 1986;9:45-60.
7. Billington BM, Leaver PK, McLeod D. Management of retinal detachment in the Wagner-Stickler syndrome. *Trans.Ophthalmol.Soc.U.K.* 1985;104 (Pt 8):875-9.
8. Maumenee IH. Vitreoretinal Degeneration As A Sign of Generalized Connective-Tissue Diseases. *Am.J.Ophthalmol.* 1979;88:432-49.
9. Jansen LMAA. Degeneratio hyaloideo-retinalis hereditaria. *Opthalmologica* 1962;144:458-64.
10. Liberfarb RM, Goldblatt A. Prevalence of mitral-valve prolapse in the Stickler syndrome. *Am.J.Med.Genet.* 1986;24:387-92.
11. Nielsen CE. Stickler's Syndrome. *Acta Ophthalmol.(Copenh).* 1981;59:286-95.
12. Wagner H. Ein bisher unbekanntes Erbleiden des Auges (Degeneratio hyaloideo-retinalis hereditaria), beobachtet im Kanton Zürich. *Klin.Monatsbl.Augenheilkd.* 1938;100:840-57.
13. Ikegawa S, Iwaya T, Taniguchi K, Kimizuka M. Retinal Detachment in Spondyloepiphyseal Dysplasia Congenita. *J.Pediatr.Orthop.* 1993;13:791-2.
14. Madigan WP, Wertz D, Cockerham GC, Thach AB. Retinal Detachment in Osteogenesis Imperfecta. *J.Pediatr.Ophthalmol.Strabismus.* 1994;31:268-9.
15. Maumenee IH, Traboulsi EI. The Ocular Findings in Kniest Dysplasia. *Am.J.Ophthalmol.* 1985;100:155-60.
16. Pemberton JW, Freeman HM, Schepens CL. Familial retinal detachment and the Ehlers-Danlos syndrome. *Arch.Ophthalmol.* 1966;76:817-24.
17. Go SL, Maugeri A, Mulder JJS, van Driel MA, Cremers FPM, Hoyng CB. Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the COL2A1 gene. *Invest.Ophthalmol.Vis.Sci.* 2003;44:4035-43.

18. Machemer R, Aaberg TM, Freeman HM, Irvine AR, Lean JS, Michels RM. An updated classification of retinal detachment with proliferative vitreoretinopathy. *Am.J.Ophthalmol.* 1991;112:159-65.

19. Richards AJ, Martin S, Yates JR, Scott JD, Baguley DM, Pope FM et al. COL2A1 exon 2 mutations: relevance to the Stickler and Wagner syndromes. *Br.J.Ophthalmol.* 2000;84:364-71.

20. Schepens CL, Marden D. Data on the natural history of retinal detachment. Further characterization of certain unilateral nontraumatic cases. *Am.J.Ophthalmol.* 1966;61:213-26.

21. Thelen U, Gerding H, Clemens S. Seasonal variations in the incidence of rhegmatogenous retinal detachment. *Ophthalmologe* 1997;94:638-41.

22. Tornquist R, Tornquist P, Stenkula S. Retinal Detachment - A Study of A Population-Based Patient Material in Sweden 1971-1981. 2. Preoperative Findings. *Acta Ophthalmol.(Copenh).* 1987;65:223-30.

23. Byer NE. What happens to untreated asymptomatic retinal breaks, and are they affected by posterior vitreous detachment? *Ophthalmology* 1998;105:1045-9.

24. Scott JD. Prevention and Perspective in Retinal Detachment. *Eye* 1989;3:491-515.

Chapter

4

Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the *COL2A1* gene

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4.1 Abstract

Purpose:
To investigate the clinical features and molecular causes of autosomal dominant rhegmatogenous retinal detachment (RRD) in two large families.

Methods:
Clinical examination and linkage analysis of both families using markers flanking the *COL2A1* gene associated with Stickler syndrome type 1, the loci for Wagner disease/erosive vitreoretinopathy (5q14.3), high myopia (18p11.31 and 12q21-q23), and nonsyndromic congenital retinal nonattachment (10q21).

Results:
Fifteen individuals from family A and 12 individuals from family B showed RRD or retinal tears with minimal (family A) or no (family B) systemic characteristics of Stickler syndrome and no ocular features of Wagner disease or erosive vitreoretinopathy. The RRD cosegregated fully with a chromosomal region harbouring the *COL2A1* gene with maximum lod scores of 6.09 (family A) and 4.97 (family B). In family B, an Arg453Ter mutation was identified in exon 30 of the *COL2A1* gene, that was previously described in a patient with classic Stickler syndrome. In family A, DNA sequence analysis revealed no mutation in the coding region and at the splice sites of the *COL2A1* gene.

Conclusions:
In two large families with RRD, linkage was found at the *COL2A1* locus. In one of these families an Arg453Ter mutation was identified, which is surprising, because all predominantly ocular Stickler syndrome cases until now have been associated with protein-truncating mutations in exon 2, an exon subject to alternative splicing. In contrast, the Arg453Ter mutation and other protein-truncating mutations in the helical domain of *COL2A1* until now have been associated with classic Stickler syndrome.

4.2 Introduction

Rhegmatogenous retinal detachment (RRD) often is associated with (pathologic) myopia and in most cases leads to visual impairment or blindness if untreated.[1;2] Early diagnosis of RRD and recognition of patients at risk improve the prognosis (see Reference 3 and the references therein). Nonsyndromic pathologic myopia (-6 D or less) in most cases occurs sporadically, but is also encountered as an autosomal dominant or X-linked trait in families.[4-7] RRD with autosomal dominant inheritance in association with myopia and vitreoretinal degeneration is usually described as a feature of Stickler syndrome or erosive vitreoretinopathy. RRD also has been reported in the original Wagner family, although less frequent.[8]

Stickler syndrome is characterized by systemic abnormalities as midfacial hypoplasia, midline cleft of the palate, sensorineural hearing loss, early progressive arthropathies, and hypermobility, in combination with ocular abnormalities, such as high myopia, abnormalities of the vitreous structure, paravascular pigmentation and possibly giant tears causing retinal detachment.[9-11] Mitral valve prolapse also has been reported.[12] These features show intra- and interfamilial variability of expression. Moreover, different types of Stickler syndrome can be distinguished based on the presence or absence of ocular abnormalities, the appearance of the vitreous, and the molecular genetic findings. Type 1 Stickler syndrome is characterized by a membranous vitreous phenotype and is caused by mutations in the *COL2A1* gene.[13-15] Type 2 Stickler syndrome exhibits a different beaded vitreous phenotype and has been associated with *COL11A1* mutations.[15-17] Nonocular Stickler syndrome type 3, with a phenotype displaying characteristic systemic abnormalities such as facial abnormalities, cleft palate, hearing loss, and arthropathies, but without high myopia, vitreoretinal degeneration, or retinal detachments, is caused by mutations in *COL11A2*.[18-20] Evidence of at least a fourth locus for Stickler syndrome has been found, as mutations in the former three known genes were not found in some Stickler families.[17;21]

Wagner disease, on the other hand, is a nonsystemic disorder in which the vitreous is optically empty, and a preretinal membrane is present in the periphery of the retina, sometimes only as a thin white circular line. A progressive complicated cataract appears in most of the patients, chorioretinal atrophy, peripheral pigment foci, and a situs inversus of the optic disc may be present.[22-24] Wagner disease has been mapped to the long arm of chromosome 5 in region 14.3 (5q14.3).[25]

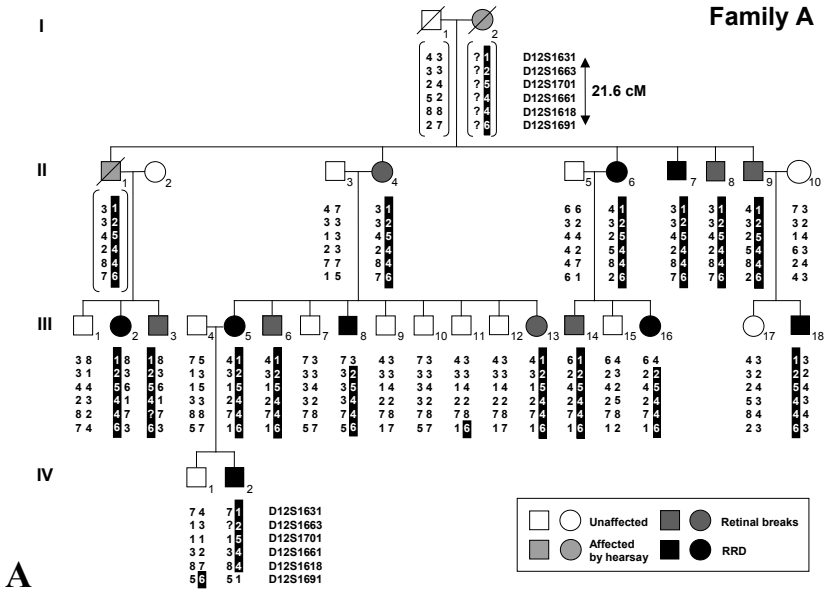
In erosive vitreoretinopathy, progressive thinning of the retinal pigment epithelium resulting in severe degeneration is the major feature. In addition, and in contrast with Wagner disease, a pronounced roped and veiled syneresis of the vitreous body with traction at lesions of the retinal pigment epithelium and frequent development of retinal detachment, both rhegmatogenous and tractional, are observed. As in Wagner disease, no systemic abnormalities are found.[26;27] The disorder maps to the same region as Wagner disease, 5q13-q14[27], suggesting that erosive vitreoretinopathy and Wagner

disease may be allelic disorders.
In this report, we present two large families with autosomal dominant RRD or retinal breaks without or with minimal systemic features, clinically different from Wagner disease, erosive vitreoretinopathy, and typical Stickler syndrome. Both families showed linkage to a genomic region containing the *COL2A1* gene. In one of the families, a stop codon mutation was found in the helical domain of the *COL2A1* gene that had been found earlier in a patient with typical Stickler syndrome.

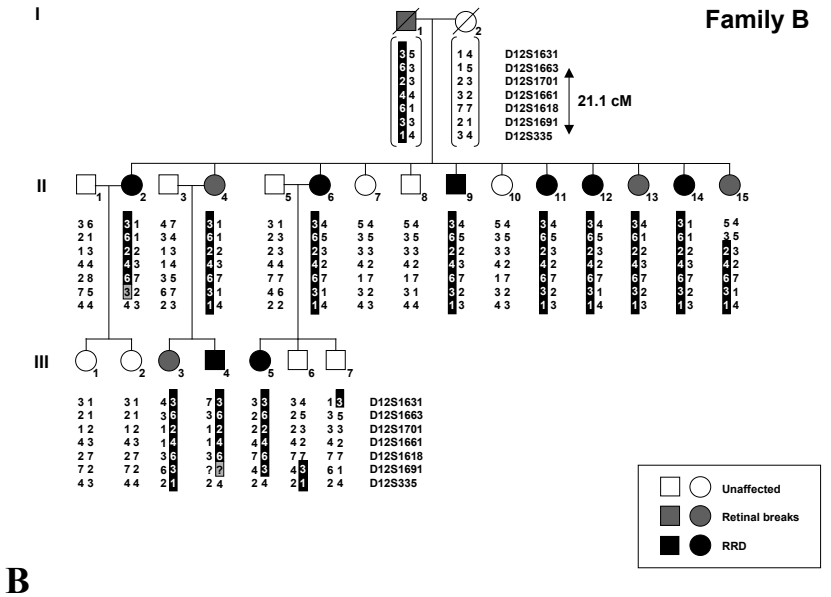
4.3 Patients and methods

Two unrelated Dutch families of white origin with autosomal dominant RRD were studied. Family A consisted of 28 individuals; family B consisted of 22 individuals (**Figure 4.1**). The study protocol followed the tenets of the Declaration of Helsinki, and informed consent was obtained from each participant or their guardians, after general approval by the Ethics Committee of the University Medical Centre Nijmegen, the Netherlands.

Figure 4.1 Haplotype analysis of families with RRD or retinal breaks with markers encompassing the *COL2A1* gene on the long arm of chromosome 12, region 13.11. (*See next page*)
The *COL2A1* gene resides between markers D12S1701 and D12S1661. The shared alleles from the at-risk haplotype are shown in black bars, marker alleles between brackets are deduced. (**A**) Family A: the boundaries of the critical interval between D12S1631 and D12S1691 are determined by recombination events in affected individuals AIII-8, AIII-16 and AIV-2. (**B**) Family B: the critical interval between D12S1663 and D12S335 is determined by recombination events in affected individuals BII-15 and BIII-5. Note that marker D12S1691 in individuals BII-2 and BIII-4 are noninformative (grey bars). Assuming that individual BIII-6 is not a nonpenetrant, the telomeric boundary is demarcated by marker D12S1691, thereby reducing the critical region to 15.8 cM. The DNA marker order and distances were derived from the Human Genome Browser (April 2002 assembly)[28] and Généthon.[29] Note the overlap of the critical intervals of families A and B.


Family A
I
II
III
IV
D12S1631
D12S1663
D12S1701
D12S1661
D12S1618
D12S1691
21.6 cM
Unaffected
Affected by hearsay
Retinal breaks
RRD

A


Family B
I
II
III
D12S1631
D12S1663
D12S1701
D12S1661
D12S1618
D12S1691
D12S335
21.1 cM
Unaffected
Retinal breaks
RRD

B

Clinical examination

An extensive clinical history of all individuals especially regarding ophthalmic, audiologic, cardiologic, and orthopaedic disorders, and current symptoms was recorded. Existing ophthalmic records of all participants and, if possible, of the deceased were collected and reviewed regarding age of onset of myopia, structure of the vitreous body, retinal breaks, retinal detachments or other abnormalities of the fundus, biometric measurements, and intraocular pressure. Clinical examination included best corrected visual acuity, slit-lamp microscopy, applanation tonography, fundoscopy including fundus photography, and Goldmann three-mirror contact glass examination. These examinations were performed in 27 individuals in family A and 22 individuals in family B by both a highly experienced medical retina specialist (CBH) and by the first author (SLG). Special attention was paid to the vitreous body structure. Axial length measurement and keratometry were performed, using ultrasound. In cases of axial length of 25 mm or longer or in cases with closed pupillae, ultrasound examination of the posterior eye was performed. All individuals with retinal detachments or retinal breaks were considered affected.

Physical examination including assessment of facial, palatal, joint, and heartsound abnormalities was prospectively performed in affected individuals who were willing to cooperate. Existing audiologic, cardiologic, and orthopaedic medical records were collected. Facial and palatal photographs were taken. The Beighton score for hypermobility of joints[30] and audiometry were assessed in six cases of family A and ten of family B.

Molecular genetic analysis

DNA was extracted from leucocytes from 10 mL of peripheral blood of all individuals, according to a protocol adapted from Miller et al.[31] Linkage analysis was performed with radioactively labelled microsatellite markers. The candidate loci were the two loci for autosomal dominant high myopia on chromosome 18p11.31 (MYP2; markers D18S52 [AFM020tf12] and D18S1154 [AFMa056ye1]) and 12q21-q23 (MYP3; markers D12S64 [MFd155a], D12S82 [AFM107xc11] and D12S317 [AFM065ye9]), the Wagner disease/erosive vitreoretinopathy locus on 5q14.3 (markers D5S428 [AFM238xf4] and D5S2094 [AFMa055td9]), the locus for nonsyndromic congenital retinal nonattachment on 10q21 (marker D10S581 [AFM287yf9]), and the genes for Stickler syndrome *COL2A1* on 12q13.11-q13.2 and *COL11A1* on 1p21.1. For *COL2A1*, residing between markers D12S1701 and D12S1661, we used the following markers (from pter to qter; genetic distances indicated): D12S1631 (AFMa288wd5) -5.8 centimorgans (cM)- D12S1663 (AFMb316xd9) -6.2 cM- D12S1701 (AFM345xf1) -1.4 cM- D12S1661 (AFMb314yh5) -4.6 cM- D12S1618 (AFMa224yg1) -3.6 cM- D12S1691 (AFM312xf5) -5.3 cM- D12S335 (AFM273vg9).[29] No marker near *COL11A1* was tested, because linkage was found at the *COL2A1* locus. DNA samples were subjected to polymerase chain reaction (PCR) amplification with a standard cycling profile of 30 cycles at 94°C, 55°C, and 72°C with 1, 2, and 1 minute(s)

respectively at each step. DNA markers were labelled by the incorporation of $\alpha[^{32}P]$-dCTP and the products were separated by electrophoresis on a 6.6% denaturing polyacrylamide gel.

Linkage analysis by calculating two-point lod scores was performed using the MLINK routine from the LINKAGE (Version 5.1) software suite.[32-34] Lod scores in both families were calculated with a presumed penetrance rate of 95% and an allele frequency of 0.001.

Mutation analysis of the *COL2A1* gene was performed by direct sequencing (BigDye Terminator on a Prism 377; Applied Biosystems, Foster City, CA USA). The entire coding region of the gene, comprising 54 exons, was amplified in 38 amplicons. Primer pairs and conditions are available on request. To ascertain mutations that could affect the splicing, at least 42 bps (average 104 bps) of the flanking intronic sequences were amplified. Twenty-one introns (introns 3-6, 9, 13, 20, 21, 24, 25, 30, 32, 35, 40, 42-48) were entirely amplified. Sequence analysis was performed on both strands of each amplicon using both forward and reverse primers.

To assess the stability of the mutant *COL2A1* messenger RNA (mRNA), Epstein-Barr virus- transformed lymphoblastoid cell lines were established from heparin blood of two affected individuals from family B. Before RNA extraction, half of the cultured cells were incubated for 4 hours with 100 μg/mL cycloheximide. In cells grown with cycloheximide, a protein synthesis inhibitor, the nonsense-mediated mRNA decay process is prevented.[35] After RNA extraction and reverse transcription-PCR (RT-PCR), a fragment of the cDNA encompassing the mutation in exon 30 was amplified using a first set of primers, 5051F (5`-tgcctggtgaaagaggacggac-3`) and 5054R (5`-ggcattccctgaagacctggag-3`), followed by a nested PCR and direct sequencing of the band of interest with primer 5053F (5`- tcaagatggtctggcaggtccc-3`) and the same reverse primer 5054R.

4.4 Results

Clinical examination

The ophthalmic examination was performed in 27 of 28 individuals from family A and in all 22 individuals from family B. One individual (AIII-3) refused prospective clinical examination, but could be considered affected because a retinal break was described in his medical files. Medical files of AI-2 and AII-1 were not available, but these family members were determined by hearsay to be affected. The medical files of individual BI-1 showed a retinal break and that person was thus considered to be affected. BI-2 had no known health problems before her death. The clinical features of all affected individuals (15 of 28 in family A, 12 of 22 in family B) and the available information about BI-1 are shown in **Table 4.1**. Refractive error comprised the whole scale of mild hypermetropia to high myopia in family A, whereas the scale was limited between no myopia and severe myopia in family B, with a tendency toward moderate or high myopia. In both families, the myopia was axial-length dependent (mean axial lengths: 24.8 mm [range 20.2-28.2 mm] in family A, 26.6 mm [range 24.9-28.7 mm] in family B). There was no specific abnormality of the vitreous body that was found in all affected individuals in both families, especially no consistent vitreal membranes or beaded strands. Only RRDs, or at least retinal breaks, were a consistent ophthalmic finding throughout the families. In family A, 11 RRDs occurred in 8 of the 15 affected family members, with an average age of first onset of RRD of 36 years (range 16-64 years). Seven of the 12 affected members of family B experienced early RRDs in nine eyes. The average age of onset of RRD in this family was 14 years (range 7-22 years). Eyes with RRDs showed a tendency to multiple (average 2; range 0-7) peripheral holes or horseshoe tears in the temporal superior and inferior quadrants in family A, whereas the periphery of the eyes of the affected in family B mostly revealed round multiple (average 8; range 1-28) retinal holes in the temporal superior quadrant. Bilateral RRDs were seen in patients AIII-5, AIII-18, AIV-2, BII-2 and BIII-4.

The history and clinical examination of all examined individuals of both families revealed no systemic abnormalities, except for five persons. Individual AII-6 had a history of surgery for a left-side cerebellar cyst and showed a sensorineural hearing defect in all frequencies of the left ear only. Thresholds at frequencies 0.25, 0.5, 1, 2, 4 and 8 kHz (thresholds more than age-related between brackets), respectively, were [50], 20, [50], [70], [100] and [110] dB hearing loss at the age of 76 years. AIII-2, at 54 years of age, had a slightly recessed chin, a symptomatic progressive low- and midfrequency sensorineural hearing loss with thresholds of [25], [42.5], [55], [50], 32.5 and 40 dB, respectively, for both ears (ADS), and symptoms of occasionally stiff fingers of both hands and pains in her left knee after long walks. AIII-5 had thresholds of 15, 17.5, [25], [30], 30 and [67.5] dB hearing loss ADS, but was asymptomatic at 53 years. AIII-6 had a noise-exposition history and at the age of 51 years showed thresholds of 12.5, 17.5, 5, 7.5, [50] and 7.5 dB hearing loss ADS. Finally, individual

BIII-4 had a transient flat nose bridge in the first decade of his life, but now has a normal facial appearance. Individuals BII-15 and BIII-3 showed a small air bone gap of 7 dB, probably related to tubal dysfunction at the time. As was true of all other patients from this family, they had a normal symmetrical age-related sensorineural threshold.

Table 4.1 Clinical features of affected individuals from family A and B

			Vitreous		Retina				Systemic phenotype[§]	
Patient	Myopia grade[*]	Significant early cataracts[¶]	Optical density	Visible structure(s) (age in yrs)	Detachment[•]	Age at onset of RRD (yrs)	Break (age in yrs)	Midfacial hypoplasia[‖]	Hearing loss (age in yrs)[#]	Articular complaints (yrs)
AII-4		-	Normal	Normal	0		+ (65)	0	-	-
AII-6	3	-	Thin	Strands	1 (postphacogenic uveitis)	60	+ (60)	0	+, asymmetrical sensorineural low-, mid- and high-frequency loss with history of a left-sided cerebellar cyst operation (76)	-
AII-7		-	Empty	Few posterior condensations	1 (postneovascular glaucoma, possibly as a result of longterm RD)	64	Not found	0	-	-
AII-8	2	-	Empty	Threads, posteriorly: membrane OD, strands OS (64)	0		+ (44)	0	-, audiologically confirmed (67)	-
AII-9	2	-	Empty	Condensations	0		+ (46)	0	-	-
AIII-2	3	+	Thin		1	35	+ (35)	1	+, progressive sensorineural low-and midfrequency loss (54)	Incidentally stiff fingers of both hands, pains in left knee after lengthy walks (51)
AIII-3	-1	+	Empty parts	Thick threads ODS (29); PVD or just primary vitreous present (30)[§]	0		+ (30)	0	-	-
AIII-5	-1	-		Veils and strands	2	45	+ (44)	0	+, sensorineural mid- and highfrequency loss (52)	Stiffness and pain during unexpected hip movements (49)
AIII-6	0	-	Normal	Threads (49)	0		+ (51)	0	+, sensorineural loss at 4 kHz with history of noise exposition (51)	-
AIII-8	3	-		Veils	1	29	+ (29)	0	-	-
AIII-13	3	-		Veils (30); threads (35)	0		+ (30)	0	-	-
AIII-14		-		Threads (45)	0		+ (40)	0	-	-
AIII-16	2	-		Veils	1	33	+ (33)	0	-	-
AIII-18	2	-	Empty	Veils (16); veils and membrane (17); collapsed vitreous, fine structured OD, thicker OS (35)	1	16	+ (15)	0	-	-

AIV-2	1	-		Midperipheral white condensations ODS, thick epiretinal fibrosis OS (16); same in OD (17); peroperatively: very thick vitreous, thick grey mass at pars plana (19)	2	16	+ (16)	0	-, audiologically confirmed (23)	-
BI-1	2				0		+ (52)		-	
BII-2	3	+	Thin	Threads (11); veils (25)	2	11	+ (11)	0	-	-
BII-4	2/3	-	Thin	Retrolental membrane (17); threads and very fine beads (40)	0		+ (17)	0	-, audiologically confirmed (42)	-
BII-6	2	-		Condensations and veils OD (17); fine threads, fine beads (39)	1	17	+ (16)	0	-, audiologically confirmed (42)	-
BII-9	2/3	+		Veils (27)	1	27	Not found	0	-, audiologically confirmed (35)	-
BII-11	3	+	Very thin OD, thicker OS,	Collapsed vitreous (?)	1	27	+ (27)	0	-, audiologically confirmed (31)	-
BII-12	3	-	Dense structure	Retrolental membrane OD (28)	1	22	+ (22)	0	-, audiologically confirmed (30)	-
BII-13	2	+		Retrolental threads (26)	0		+ (10)	0	-, audiologically confirmed (29)	-
BII-14	2	-	Normal	Normal	1	16	+ (16)	0	-, audiologically confirmed (26)	-
BII-15	1	-	Empty	Collapsed vitreous ODS with beaded threads OD (22)	0		+ (22)	0	-, audiologically confirmed but small air bone gap of 7 dB probably related to tubal dysfunction (21)	-
BIII-3	2	-	Very thin	Branched threads, membranelike structure more posteriorly (19)	0		+ (19)	0	-, audiologically confirmed but small air bone gap of 7 dB probably related to tubal dysfunction (24)	-
BIII-4	0	-		Thick veils (9); threads with fine beads and retrolental membrane, OS less threads than OD, not PVD (10)	1 (after blunt trauma)	9	+ (9)	1	-, audiologically confirmed (12)	-
BIII-5	2/3	-	Thin	Veils (6); thick threads OD(9); some retrolental threads ODS (13)	1	6	+ (6)	1	-	-

+ present; - absent; RRD = Rhegmatogenous retinal detachment, PVD = Posterior vitreous detachment

*: Myopia grade prior to RRD or retinal break (using spherical equivalents): -1 mild hypermetropia, 0 emmetropia, 1 mild (0 D to -1.5D), 2 moderate (-1.5 D to –6.0 D), 3 high myopia (-6.0 D or more)

¶: Nontraumatic cataracts, at less than 40 years of age

•: 0 absent, 1 unilateral, 2 bilateral

§: None of the examined patients showed cleft palate or joint hypermobility (Beighton score ≥4)

||: 0 absent, 1 mild, 2 moderate, 3 severe

#: Hearing loss more than age-related (>P95, ISO7029 040996)

$: Retrospective data only, examined elsewhere, and examining doctor deceased

DNA analysis

We excluded the involvement of the MYP2 and MYP3 loci for autosomal dominant high myopia, as well as the loci for Wagner/erosive vitreoretinopathy and nonsyndromic congenital retinal nonattachment by linkage analysis in family A (**Table 4.2**).

Table 4.2 Lodscores for Two-Point Linkage analysis in RRD families A and B

Family A									
Locus	**Location**	**Marker**	**Recombination fraction**						
			0.000	**0.010**	**0.050**	**0.100**	**0.200**	**0.300**	**0.400**	**0.500**
MYP2	18p11.31	D18S52	-11.13	-6.00	-3.16	-1.85	-0.69	-0.22	-0.04	0.00
		D18S1154	-12.01	-7.29	-3.71	-2.03	-0.60	-0.07	0.07	0.00
MYP3	12q21-q23	D12S64	-4.04	-1.17	-0.11	0.35	0.56	0.40	0.14	0.00
		D12S82	-∞	-10.43	-6.67	-4.54	-2.16	-0.93	-0.29	0.00
		D12S317	-8.31	-4.88	-3.01	-1.78	-0.62	-0.17	-0.03	0.00
WGN1/ ERVR	5q14.3	D5S428	-9.18	-5.21	-2.54	-1.32	-0.30	0.02	0.07	0.00
		D5S2094	-8.31	-4.33	-2.42	-1.49	-0.63	-0.24	-0.05	0.00
NCRNA	10q21	D10S581	0.86	0.84	0.74	0.63	0.40	0.20	0.05	0.00
COL2A1	12q13.11-q13.2	D12S1631	-5.38	2.00	3.01	3.10	2.63	1.82	0.84	0.00
		D12S1663	4.91	4.82	4.45	3.97	2.97	1.91	0.82	0.00
		D12S1701	5.79	5.69	5.28	4.75	3.63	2.41	1.11	0.00
		D12S1661	6.09	5.98	5.56	5.01	3.83	2.56	1.19	0.00
		D12S1618	5.79	5.69	5.28	4.75	3.63	2.42	1.11	0.00
		D12S1691	-0.81	1.55	2.30	2.46	2.17	1.53	0.69	0.00
Family B									
Locus	**Location**	**Marker**	**Recombination fraction**						
			0.000	**0.010**	**0.050**	**0.100**	**0.200**	**0.300**	**0.400**	**0.500**
COL2A1	12q13.11-q13.2	D12S1631	-3.23	1.67	2.28	2.37	2.03	1.39	0.54	0.00
		D12S1663	-1.93	2.89	3.27	3.16	2.56	1.72	0.69	0.00
		D12S1701	2.62	2.58	2.38	2.13	1.59	0.99	0.37	0.00
		D12S1661	1.72	1.69	1.57	1.41	1.07	0.68	0.26	0.00
		D12S1618	4.97	4.89	4.55	4.12	3.17	2.09	0.86	0.00
		D12S1691	0.42	0.47	0.58	0.62	0.54	0.35	0.11	0.00

In both families, highly polymorphic DNA markers flanking the *COL2A1* gene showed co-segregation with the disease (**Table 4.2**). In family A, the critical region is demarcated by markers D12S1631 and D12S1691 (interval: 21.6 cM, 28 Mb), based on crossovers observed in the affected individuals AIII-8, AIII-16 and AIV-2 (**Figure 4.1A**). The maximum lod score, 6.09, was detected for marker D12S1661 at a

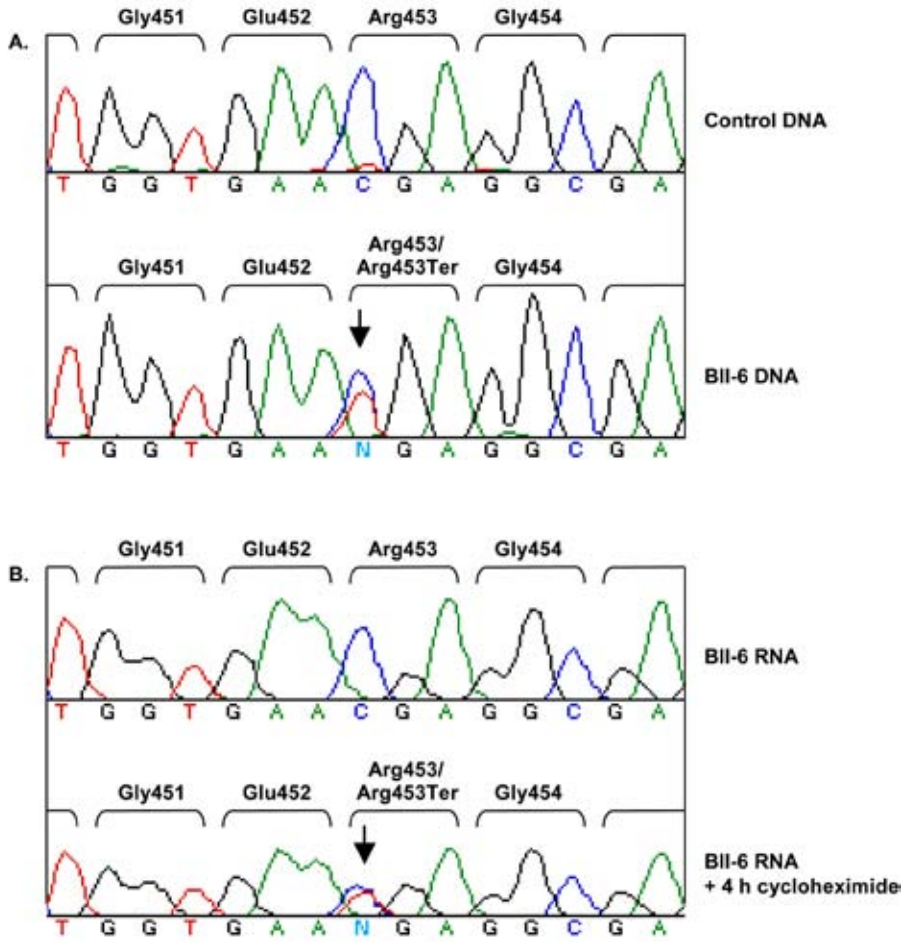


Figure 4.2 *COL2A1* mutation analysis in patient BII-6
(**A**) DNA sequence analysis of part of exon 30 (previously denoted as exon 28) of the *COL2A1* gene. Top: sequence of a control individual; bottom: sequence of the clinically affected individual BII-6, carrying a heterozygous C-to-T transition resulting in an Arg453Ter mutation. (**B**) RNA analysis of the Arg453Ter mutation in patient BII-6. Top: sequence of the cDNA of the patient. Because of mRNA instability, the transcript of the mutant allele was not detectable. Bottom: sequence of the cDNA of the patient obtained after incubation of lymphoblastoid cells in a medium containing cycloheximide, which prevents nonsense-mediated mRNA decay. Sequence analysis shows presence of transcripts from both the normal and the mutant allele.

recombination fraction (θ) of 0.0. In family B, a linked chromosomal region of 21.1 cM (26 Mb) was delimited by markers D12S1663 and D12S335, based on recombination events observed in affected individuals BII-15 and BIII-5 (**Figure 4.1B**). A maximal lod score of 4.97 was found for marker D12S1618 at $\theta = 0.0$. Assuming that the healthy individual BIII-6 is not a nonpenetrant, the telomeric boundary is demarcated by marker D12S1691, thereby reducing the critical region to 15.8 cM (16 Mb).
In family A, analysis of all 54 exons and flanking intronic regions of *COL2A1* failed to identify a mutation in the coding region or at the splicing sites of the gene.
In family B, mutation analysis of the *COL2A1* gene showed a C-to-T transition in exon 30 (previously denoted as exon 28), resulting in a change of codon CGA of Arg 453 for a stop codon (**Figure 4.2A**). Ninety-six ethnically matched controls did not show this mutation. Analysis of RNA extracted from lymphoblastoid cells grown with and without cycloheximide from patient BII-6 showed stability of mutant RNA only in cells grown with cycloheximide (**Figure 4.2B**), strongly suggesting that the *COL2A1* mRNA carrying the Arg453Ter mutation is unstable.

4.5 Discussion

In this study, we report on a family (B) that shows autosomal dominant RRD associated with a mutation in the triple helical domain of the *COL2A1* gene. The mutation, Arg453Ter, has been described in a sporadic patient with Stickler syndrome, who had had such classic features as cleft palate, midfacial hypoplasia, sensorineural hearing loss, joint laxity, and joint pains since the second decade, besides high myopia, vitreoretinal degeneration with a typical type I vitreous anomaly (William G. Cole, personal communication, 2002), retinal breaks, and retinal detachment in the first decade.[36] Protein-truncating *COL2A1* mutations are commonly found in patients with Stickler syndrome.

The phenotype observed in patients in family B however, was different from the classic Stickler syndrome. In all 12 RRD patients -even in the eldest generation- cleft palate, joint laxity, joint pains or sensorineural hearing loss were absent, whereas these symptoms were already present in the reported 23-year-old patient.[36]

Use of Snead's criteria- that is, a congenital vitreous anomaly (type 1: membranous, or type 2: fibrillar, beaded) and any three of the following: (a) myopia with onset before 6 years of age, (b) RRD or paravascular pigmented lattice degeneration, (c) joint hypermobility with abnormal Beighton score, either with or without radiological evidence of joint degeneration, (d) audiometric confirmation of sensorineural hearing defect, and (e) midline clefts[15] in this family also indicates that patients of family B are clinically different from patients with classic Stickler syndrome. First of all, except four patients in whom membranelike vitreous abnormalities were found (individuals BII-4, BII-12, BIII-3 and BIII-4), no members of family B revealed a vitreous consistent with a type I or type II Stickler vitreous. In BII-4, the membranelike structure was absent 23 years later, possibly due to degeneration of the membrane.

Furthermore, myopia, if present, was not always present before 6 years of age (data not shown). Joint hypermobility with abnormal Beighton score and midline clefts were not observed, and were not anamnestically present during childhood. Audiometric results were not suggestive of Stickler syndrome, and in both BIII-3 and BII-15 were most probably due to tubal dysfunction at the time of examination. Moreover, although none of the affected individuals from family B had joint pains, according to surveys, 70% of patients with Stickler syndrome have joint pains before 20 years of age.[37]

Family A, in which the underlying genetic defect also cosegregates with the *COL2A1* locus, although no mutation could be detected in the coding region and at the splice sites of the gene, also does not meet the classic criteria nor Snead's criteria for Stickler syndrome. First, the vitreous of all family members does not comprise consistent membrane- or threadlike abnormalities, though a thin vitreous body was present in several family members. In addition, a whole range of refractive errors between mild hypermetropia and high myopia was found in all affected individuals. No cleft palates were found, and though sensorineural hearing loss was found in four individuals,

it was not typical of Stickler syndrome. Two of these hearing defects are explained by noise exposition (AIII-6) and a left-side cerebellar cyst that had been surgically removed (AII-6). AIII-5 had an asymptomatic mid- and high-frequency hearing loss of 10 dB more than age-related hearing loss and only one individual, AIII-2, had a symptomatic, progressive sensorineural hearing defect of 23 dB more than age-related hearing loss. However, the defect in this patient affected the low- and midfrequencies, although in patients with Stickler syndrome, the hearing impairment generally involves the high frequencies and shows no more progression than is associated with normal aging.[11;38] Joint hypermobility was not present during childhood and was not observed in those who had been examined. Except in patient AIII-2 at 51 years of age and patient AIII-5 who had pains in the left hip region during unexpected hip movements at age 49 years, no joint pains were found in patients of family A. Radiography of patient AIII-5 showed a moderate arthrosis of and reduced joint space in her left hip at the age of 52 years.

Previously described families with predominantly ocular Stickler syndrome invariably showed a type I vitreous anomaly, and all had mild to moderate systemic abnormalities, be it that these were present in only about half of the examined family members.[39] Also, if we consider each of these families as one unit, the abnormalities found in one family altogether invariably led to a complete Stickler syndrome diagnosis by Snead's criteria. This "family diagnosis" could not be made in each of our families, when taking into account all clinical abnormalities. There also was no consistent type I vitreous anomaly.

We think that patients of families A and B do not have Wagner disease, because strongly progressive juvenile cataract and inverted papilla, preretinal membranes or peripheral circular lines were not present. Moreover, of the 15 affected members of family A, only four showed an optically empty vitreous body and one had empty parts in the vitreous body, whereas in family B only 1 of 12 affected individuals showed an optically empty vitreous body. In contrast, this was invariably present in patients from the original Wagner family.[22]

In conclusion, our results suggest that the patients in families A and B had an atypical form of predominantly ocular Stickler syndrome with RRD as the main clinical feature.

An important difference between both families is that retinal breaks and detachments in family B occurred at younger ages, mostly in the second and third decades, whereas in family A they mostly appeared in the fourth and fifth decades and, in a few cases, even later (individuals AII-4, AII-6, AII-7 and AIII-6; **Table 4.1**).

Until recently, it seemed that *COL2A1* gene mutations could be associated with type 1 vitreous, whereas C*OL11A1* gene mutations were responsible for type 2 vitreous. Discussion of the role of the vitreous types in predicting the mutated gene, however, was recently published.[40;41] In fact, a Stickler family with a type I vitreous had linkage to *COL11A1*[40], whereas in two Stickler families with a type II vitreous, *COL11A1* gene mutations were excluded.[21] Earlier posterior vitreoretinal detachment was suggested

to have caused these phenotypes, because in two families conversion from vitreous phenotype 2 into 1 was observed.[41] Our data also contradict the hypothesis that all *COL2A1* mutations are associated with a type I vitreous.

The most interesting result of this study, however, is the identification of a *COL2A1* exon-30 protein-truncating mutation (Arg453Ter), previously identified in a patient with classic Stickler syndrome[36], in a large family with an atypical form of predominantly ocular Stickler syndrome.

Collagen molecules are typically composed of three polypeptide chains (α-chains) that form a triple helix. A characteristic repetitive amino acid sequence, glycine-X-Y, is important for maintaining this helical structure. Three identical α1(II) chains, encoded by the *COL2A1* gene, constitute collagen II, the main collagen in cartilage and vitreous. Moreover, α1(II) chains participate in the formation of collagen V/XI in combination with α1(XI) and α2(XI) chains in the cartilage, and α1(XI) and α2(V) chains in the vitreous.[42]

The *COL2A1* gene is involved in several autosomal dominant disorders.[43] A variety of cartilage disorders, such as achondrogenesis, spondyloepiphyseal dysplasia and Kniest dysplasia, are caused by missense mutations in *COL2A1*, generally changing one of the glycine residues of the triple helical structure, or by small in-frame deletions.[43] All these mutations probably disrupt normal collagen II and collagen V/XI structure through a dominant negative mechanism.

On the contrary, all *COL2A1* mutations described in patients with Stickler syndrome (References 35, 36 and references therein) with a few exceptions[44;45] lead to a premature termination codon. Some authors demonstrated that mutant mRNAs in patients with Stickler syndrome undergo nonsense-mediated mRNA decay, resulting in *COL2A1* haploinsufficiency.[45;46] Haploinsufficiency of α1(II) chain molecules could affect collagen II production or, more likely, will disturb the stochiometry of V/XI collagen.

The discovery of premature termination mutations in exon 2 of the *COL2A1* gene in all families with predominantly ocular Stickler syndrome[39] led to the speculation that exon 2 null mutations merely give rise to ocular abnormalities, because exon 2 is subject to alternative splicing and is predominantly present in fetal and adult vitreous mRNA, but is absent in mature cartilage mRNA. However, this explanation cannot apply to our families. In fact, no mutations were found in exon 2 of the *COL2A1* gene in either family, whereas the Arg453Ter mutation in family B was located in the *COL2A1* helical domain of the gene.

RNA analysis in a patient in family B suggests that, as in typical Stickler syndrome[45;46], haploinsufficiency underlies the disease. Although clinical variability in Stickler syndrome is very high, this does not satisfactorily account for the absence of systemic features in as large a family as family B.

As clinical variability in Stickler syndrome can generically be attributed to modifier factors, an intriguing hypothesis in our case would be that a transacting modifier factor is located in the vicinity of the *COL2A1* locus, and that a favourable modifier

allele cosegregates in family B with the Arg453Ter *COL2A1* mutation, resulting in the relatively mild phenotype. However, it is worthwhile to note that family B belongs to a relatively closed religious community. It is therefore possible that, more broadly, individuals of this family share a common "favourable" genetic background, due to one or more traits, that can reside everywhere in the genome.
In family A no *COL2A1* mutation was found in the coding sequence, at the splice sites, or in the 21 introns of the gene that have been entirely sequenced. Whether the disease in family A follows a mechanism similar to that in family B remains to be elucidated.

4.6 Acknowledgments

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References

1. Risk factors for idiopathic rhegmatogenous retinal detachment. The Eye Disease Case-Control Study Group. *Am.J.Epidemiol.* 1993;137:749-57.
2. Ivanisevic M. The natural history of untreated rhegmatogenous retinal detachment. *Ophthalmologica* 1997;211:90-2.
3. La Heij EC, Hendrikse F. [Retinal detachments and retinal surgery]. *Ned.Tijdschr. Geneeskd.* 1999;143:781-5.
4. Young TL, Ronan SM, Alvear AB, Wildenberg SC, Oetting WS, Atwood LD et al. A second locus for familial high myopia maps to chromosome 12q. *Am.J.Hum.Genet.* 1998;63:1419-24.
5. Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS et al. Evidence that a locus for familial high myopia maps to chromosome 18p. *Am.J.Hum. Genet.* 1998;63:109-19.
6. Naiglin L, Gazagne C, Dallongeville F, Thalamas C, Idder A, Rascol O et al. A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. *J.Med.Genet.* 2002;39:118-24.
7. Schwartz M, Haim M, Skarsholm D. X-linked myopia: Bornholm eye disease. Linkage to DNA markers on the distal part of Xq. *Clin.Genet.* 1990;38:281-6.
8. Graemiger RA, Niemeyer G, Schneeberger SA, Messmer EP. Wagner vitreoretinal degeneration. Follow-up of the original pedigree. *Ophthalmology* 1995;102:1830-9.
9. Stickler GB. Hereditary progressive arthro-ophthalmopathy. *Mayo Clin.Proc.* 1965;40:433-55.
10. Stickler GB, Pugh DG. Hereditary progressive arthro-ophthalmopathy II. Additional observations on vertebral abnormalities, a hearing defect, and a report of a similar case. *Mayo Clin.Proc.* 1967;42:495-500.
11. Lucarini JW, Liberfarb RM, Eavey RD. Otolaryngological manifestations of the Stickler syndrome. *Int.J.Pediatr.Otorhinolaryngol.* 1987;14:215-22.
12. Liberfarb RM, Goldblatt A. Prevalence of mitral-valve prolapse in the Stickler syndrome. *Am.J.Med.Genet.* 1986;24:387-92.
13. Snead MP, Payne SJ, Barton DE, Yates JR, al Imara L, Pope FM et al. Stickler syndrome: correlation between vitreoretinal phenotypes and linkage to COL 2A1. *Eye* 1994;8 (Pt 6):609-14.
14. Snead MP, Yates JR, Pope FM, Temple IK, Scott JD. Masked confirmation of linkage between type 1 congenital vitreous anomaly and COL 2A1 in Stickler syndrome. *Graefes Arch.Clin.Exp.Ophthalmol.* 1996;234:720-1.
15. Snead MP, Yates JR. Clinical and Molecular genetics of Stickler syndrome. *J.Med. Genet.* 1999;36:353-9.

16. Richards AJ, Yates JR, Williams R, Payne SJ, Pope FM, Scott JD et al. A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in alpha 1 (XI) collagen. *Hum.Mol.Genet.* 1996;5:1339-43.

17. Martin S, Richards AJ, Yates JR, Scott JD, Pope M, Snead MP. Stickler syndrome: further mutations in COL11A1 and evidence for additional locus heterogeneity. *Eur. J.Hum.Genet.* 1999;7:807-14.

18. Brunner HG, van Beersum SE, Warman ML, Olsen BR, Ropers HH, Mariman EC. A Stickler syndrome gene is linked to chromosome 6 near the COL11A2 gene. *Hum. Mol.Genet.* 1994;3:1561-4.

19. Vikkula M, Mariman EC, Lui VC, Zhidkova NI, Tiller GE, Goldring MB et al. Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. *Cell* 1995;80:431-7.

20. Sirko-Osadsa DA, Murray MA, Scott JA, Lavery MA, Warman ML, Robin NH. Stickler syndrome without eye involvement is caused by mutations in COL11A2, the gene encoding the alpha2(XI) chain of type XI collagen. *J.Pediatr.* 1998;132:368-71.

21. Wilkin DJ, Mortier GR, Johnson CL, Jones MC, De Paepe A, Shohat M et al. Correlation of linkage data with phenotype in eight families with Stickler syndrome. *Am.J.Med.Genet.* 1998;80:121-7.

22. Wagner H. Ein bisher unbekanntes Erbleiden des Auges (Degeneratio hyaloideo-retinalis hereditaria), beobachtet im Kanton Zürich. *Klin.Monatsbl.Augenheilkd.* 1938;100:840-57.

23. Böhringer HR, Dieterle P, Landolt E. Zur Klinik und Pathologie der Degeneratio hyaloideo-retinalis hereditaria (Wagner). *Opthalmologica* 1960;139:330-8.

24. Jansen LMAA. Degeneratio hyaloideo-retinalis hereditaria. *Opthalmologica* 1962;144:458-64.

25. Perveen R, Hart-Holden N, Dixon MJ, Wiszniewski W, Fryer AE, Brunner HG et al. Refined genetic and physical localization of the Wagner disease (WGN1) locus and the genes CRTL1 and CSPG2 to a 2- to 2.5-cM region of chromosome 5q14.3. *Genomics* 1999;57:219-26.

26. Brown DM, Kimura AE, Weingeist TA, Stone EM. Erosive vitreoretinopathy. A new clinical entity. *Ophthalmology* 1994;101:694-704.

27. Brown DM, Graemiger RA, Hergersberg M, Schinzel A, Messmer EP, Niemeyer G et al. Genetic linkage of Wagner disease and erosive vitreoretinopathy to chromosome 5q13-14. *Arch.Ophthalmol.* 1995;113:671-5.

28. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM et al. The human genome browser at UCSC. *Genome Res.* 2002;12:996-1006.

29. Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A et al. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996;380:152-4.

30. Rikken-Bultman DG, Wellink L, van Dongen PW. Hypermobility in two Dutch school populations. *Eur.J.Obstet.Gynecol.Reprod.Biol.* 1997;73:189-92.

31. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16:1215.

32. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc.Natl.Acad.Sci.U.S.A* 1984;81:3443-6.

33. Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am.J.Hum.Genet.* 1984;36:460-5.

34. Lathrop GM, Lalouel JM, White RL. Construction of human linkage maps: likelihood calculations for multilocus linkage analysis. *Genet.Epidemiol.* 1986;3:39-52.

35. Bateman JF, Freddi S, Lamande SR, Byers P, Nasioulas S, Douglas J et al. Reliable and sensitive detection of premature termination mutations using a protein truncation test designed to overcome problems of nonsense-mediated mRNA instability. *Hum. Mutat.* 1999;13:311-7.

36. Wilkin DJ, Liberfarb R, Davis J, Levy HP, Cole WG, Francomano CA et al. Rapid determination of COL2A1 mutations in individuals with Stickler syndrome: analysis of potential premature termination codons. *Am.J.Med.Genet.* 2000;94:141-8.

37. Stickler GB, Hughes W, Houchin P. Clinical features of hereditary progressive arthro-ophthalmopathy (Stickler syndrome): a survey. *Genet.Med.* 2001;3:192-6.

38. Szymko-Bennett YM, Mastroianni MA, Shotland LI, Davis J, Ondrey FG, Balog JZ et al. Auditory dysfunction in Stickler syndrome. *Arch.Otolaryngol.Head Neck Surg.* 2001;127:1061-8.

39. Richards AJ, Martin S, Yates JR, Scott JD, Baguley DM, Pope FM et al. COL2A1 exon 2 mutations: relevance to the Stickler and Wagner syndromes. *Br.J.Ophthalmol.* 2000;84:364-71.

40. Parentin F, Sangalli A, Mottes M, Perissutti P. Stickler syndrome and vitreoretinal degeneration: correlation between locus mutation and vitreous phenotype. Apropos of a case. *Graefes Arch.Clin.Exp.Ophthalmol.* 2001;239:316-9.

41. McLeod D, Black GC, Bishop PN. Vitreous phenotype: genotype correlation in Stickler syndrome. *Graefes Arch.Clin.Exp.Ophthalmol.* 2002;240:63-5.

42. Mayne R, Brewton RG, Mayne PM, Baker JR. Isolation and characterization of the chains of type V/type XI collagen present in bovine vitreous. *J.Biol.Chem.* 1993;268:9381-6.

43. Kuivaniemi H, Tromp G, Prockop DJ. Mutations in fibrillar collagens (types I, II, III, and XI), fibril- associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. *Hum.Mutat.* 1997;9:300-15.

44. Ballo R, Beighton PH, Ramesar RS. Stickler-like syndrome due to a dominant negative mutation in the COL2A1 gene. *Am.J.Med.Genet.* 1998;80:6-11.

45. Richards AJ, Baguley DM, Yates JR, Lane C, Nicol M, Harper PS et al. Variation in the vitreous phenotype of Stickler syndrome can be caused by different amino acid substitutions in the X position of the type II collagen Gly-X-Y triple helix. *Am.J.Hum. Genet.* 2000;67:1083-94.

46. Freddi S, Savarirayan R, Bateman JF. Molecular diagnosis of Stickler syndrome: a COL2A1 stop codon mutation screening strategy that is not compromised by mutant mRNA instability. *Am.J.Med.Genet.* 2000;90:398-406.

Chapter

5

Exclusion of *COL2A1* gene involvement in 12 small families with nonsyndromic rhegmatogenous retinal detachment

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5.1 Introduction

Rhegmatogenous retinal detachment (RRD) is a frequent phenomenon which, if untreated, generally leads to blindness.[1] Epidemiologic studies have estimated the incidence of RRD as 1:10,000 per individual per year in Caucasians.[2-5] RRD occurs predominantly in individuals between 40 and 70 years of age which have no family history for retinal detachment.[6] This hampers the identification of individuals at risk, who could benefit from preventive treatments or improved prognosis of surgery by an early diagnosis.

Known risk factors for RRD are (high) myopia (-1.00 D to - 3.00 D: 4-fold risk; < - 3.00 D: 10-fold risk), lattice degeneration, posterior vitreous detachment and peripheral retinal degeneration.[7-10] Moreover, cataract extraction and traumas can significantly increase the risk for RRD.[6;11]

In isolated patients, RRD can be considered to be a multifactorial disorder. In a recent study, Go et al. demonstrated a higher incidence of RRD independent of age, sex and myopia in siblings and offspring of patients with RRD.[5] Siblings of subjects with nonsyndromic RRD exhibited a three times increased frequency of RRD compared to siblings of unaffected subjects. These findings point to the presence of genetic factors predisposing to nonmendelian RRD, but to date none of these are known.

RRD also occurs in specific syndromes (e.g. in Stickler syndrome) and vitreoretinopathies (e.g. Wagner disease). For some of these monogenic disorders, the genetic bases have been elucidated. Most cases of Stickler syndrome are caused by mutations in the *COL2A1* and *COL11A1* genes, both genes encoding collagen molecules which are essential components of the vitreous.[12-18] Mutations in the *COL11A2* gene, which is not expressed in the vitreous, have been reported in the non-ocular Stickler syndrome phenotype.[19;20] Moreover, exclusion of linkage to *COL2A1* and *COL11A1* in several families with the full Stickler phenotype indicated the involvement of other yet unknown genes in this syndrome.[14;17]

In the last years, a number of families have been described which showed RRD with absent or minimal systemic features typical of Stickler syndrome, and which were associated with mutations in the *COL2A1* gene.[18;21-27] Most of these mutations were located in exon 2 of *COL2A1*, which is subject to alternative splicing.[21-26] The *COL2A1* mRNA variant containing exon 2 is specifically expressed in the vitreous, thereby explaining the phenotype of patients in these families in which clinical features are restricted to the eye. In a previous study, we described two large families with nonsyndromic autosomal dominant rhegmatogenous retinal detachment (adRRD).[18] Candidate gene analysis showed linkage with a chromosomal region harbouring the *COL2A1* gene. In one of these families, *COL2A1* mutation analysis of genomic DNA and cDNA failed to identify a pathologic change.[18] However, in the other family, a p.Arg453X mutation, previously described in a patient with Stickler syndrome, was found in exon 30 of the *COL2A1* gene.[18] The location of the mutation in this family

was surprising, due to the phenotype exhibited by the 12 affected members of this family, none of which presented with systemic features typical of Stickler syndrome. In order to explain our findings, we hypothesized the presence of a modifier locus, acting in trans, in the vicinity of the *COL2A1* locus. In this way, a favourable allele could co-segregate in the family in combination with the mutation. The findings in family B were unprecedented and raised the question if mutations in the *COL2A1* gene could underlie a higher number of adRRD cases than expected based on previous knowledge.

In this study, we investigated 12 small families with RRD. Most of these families exhibit a pattern of inheritance suggestive of a mendelian autosomal dominant trait. Linkage data and mutation analysis excluded the involvement of the *COL2A1* gene in the majority if not all of these families, suggesting that mutations in the *COL2A1* gene do not play a major role in familial RRD.

5.2 Materials & methods

Family recruitment and clinical investigations
Patients with RRD ascertained at the Department of Ophthalmology of the Radboud University Nijmegen Medical Centre between 2000 and 2003 were assessed for familial occurrence of RRD. Twelve families with RRD were anamnestically recognized and included in the present study (**Figure 5.1**). In total, 113 family members gave informed consent and had blood drawn for genetic analysis. Clinical charts of cooperating family members were reviewed. When necessary, further clinical examinations were performed. Clinical investigations included medical history (with special attention for ophthalmologic, audiologic, cardiac and orthopaedic disorders), visual acuity and intraocular pressure measurement, slit lamp biomicroscopy, fundoscopy, binocular contactglass examination and a physical examination including a Beighton score assessment for hypermobility where appropriate.
Individuals were considered affected when a retinal break and/or a rhegmatogenous retinal detachment was found or described. All others were considered unaffected.

Molecular genetic analysis
From all participating family members, blood samples were taken and DNA was extracted according to a protocol adapted from Miller et al.[28]
The proband of each family with RRD underwent *COL2A1* exon 2 mutation analysis by direct sequencing (BigDye Terminator on a Prism 377; Applied Biosystems, Foster City,CA) of exon 2 and intron-exon boundaries (primers and conditions available on request).
Linkage analysis was performed on all 12 families with RRD using fluorescent-labelled microsatellite markers. Two markers proximal to the *COL2A1* gene [D12S85 (AFM122xf6) and D12S1701 (AFM345xf1)] and two distal markers [D12S1661 (AFMb314yh5) and D12S339 (AFM294wc5)] were chosen. DNA fragments were amplified using a GeneAmpPCR buffer and AmpliTaq Gold (Applied Biosystems), with 2.0 mM $MgCl_2$ for all markers except D12S1701, which required 3.0 mM $MgCl_2$. PCR conditions included a first round of 15 cycles with a standard cycling profile (94°C x 15", 55°C x 15", 72°C x 30"), followed by a second round of 25 cycles with a lower denaturating temperature (89°C). PCR samples were pooled and analysed using a 3730 DNA Analyzer (Applied Biosystems).
In a few families, a polymorphic change in the *COL2A1* gene (c.504C>A in family W00-428; c.2195-55T>CG in family W02-168, W03-032, and W03-053 [RNA accession no. NM_001844; A of the ATG transcription starting codon as nucleotide 1]) was used as an additional marker.
Two-point linkage analysis was performed using the MLINK routine of the LINKAGE program package Version 5.2. RRD was analyzed as an autosomal dominant trait with incomplete age-dependent penetrance and a gene frequency of 0.0001. The

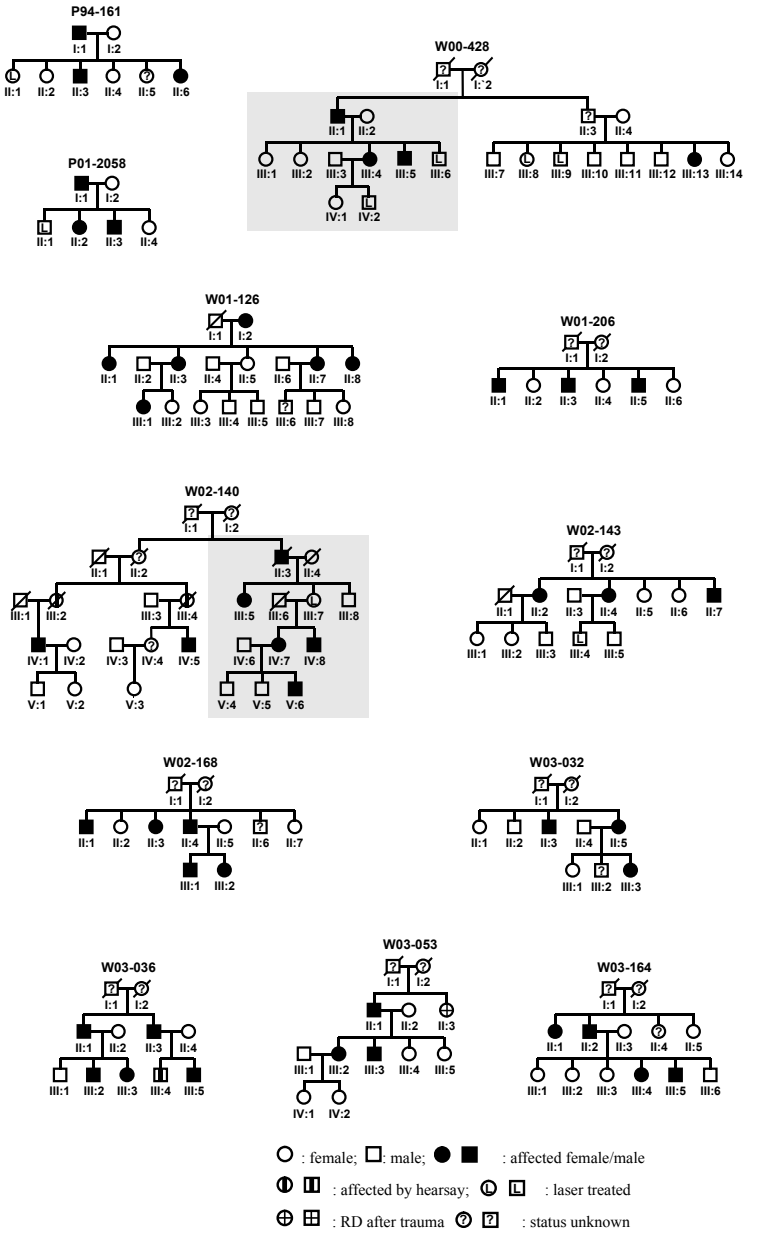

Figure 5.1 Pedigrees of the 12 families segregating RRD described in this study

penetrance was defined as the probability P (affected before current age), which is a cumulative distribution function of the age of onset. We assumed a penetrance of zero for individuals up to 5 years of age, and an age of onset uniformly distributed with a maximum penetrance of 0.95 at age 75. Age classes were (6-10), (11-15), etc. The penetrance P was calculated at the midpoint of each age class as P = [0.95/(75-5)] x (age-5). Because of the high occurrence of retinal detachment cases of multifactorial (non-mendelian) origin in the population, phenocopies were also introduced in this model. Based on the estimated cumulative lifetime risk of RRD as a function of age reported by Go et al.[5], the probability of phenocopies was considered zero up to 30 years of age, reaching a relative maximum of 0.005 at 65 years of age, and a maximum of 0.03 at 85 years of age. The age-dependent distribution of the probability of phenocopies F was described by the equations F1 = [0.005/(65-30)] x (age-30) from 0 to 65 years, and F2 = [(0.03-0.005)/(85-65)] x (age-65) + 0.005 from 65 to 85 years of age.

Probands of family P01-2058 and W02-140 were screened for *COL2A1* mutations by direct sequencing of all 54 exons and exon-intron boundaries of the *COL2A1* gene (primers and conditions available on request).

5.3 Results

Clinical features in patients with RRD
Clinical findings in probands of all families included in this study are summarized in **Table 5.1**, and are representative for the findings in these families. In total, 48 affected individuals were ascertained. In a few cases, affection status could not be established because preventive laser treatment had been performed (P94-161:II-1; W00-428:II-6 and III-2; P01-2058:II-1; W02-140:II-3; W02-143:III-4), because RRD had occurred after a trauma (W03-053:II-3) or because of incompleteness of clinical information (P94-161:II-5; W01-126:III-6; W02-168:II-6; W03-032:III-2; W03-036:III-4 [affected by hearsay]; W03-164:II-4) (**Figure 5.1**). Information on deceased parents in the first generation was generally not available. Affected individual III-2 in family W02-168 refused cooperation. In families W00-428 and W02-140, only a branch of the family (in grey box in **Figure 5.1**) entered this study.
In none of the families a typical RRD-causing syndrome was identifiable.

Mendelian inheritance of RRD
Ten out of 12 analyzed pedigrees showed a pattern of RRD cases suggestive of a dominant mode of inheritance (**Figure 5.1**). However, in family W01-126 all affected individuals in three generations are females. In the absence of male to male transmission, dominant X-linked inheritance can not be excluded. In one pedigree (W01-206), all affected individuals belong to the same generation and are males, therefore indicating a possible recessive or X-linked transmission of the disorder. Due to the uncertain status of individual III:4, who underwent laser treatment, also in pedigree W02-143 a recessive mode of transmission cannot be excluded.

Table 5.1 Clinical features in probands of the 12 families with RRD included in this study (*See next page*)
+ present, - absent, RRD = Rhegmatogenous retinal detachment, PVD = Posterior vitreous detachment, MH = macular hole, HS = horseshoe shaped defect, RH = round hole, Lattice = peripheral lattice degeneration
***** myopia grade prior to RRD or retinal break (using spherical equivalents): -1 mild hypermetropia, 0 emmetropia, 1 mild (0 D to -1.5 D), 2 moderate (-1.5 D to -6.0 D), 3 high myopia (-6.0 or more)
†: Nontraumatic nuclear cataract at 42 yrs of age.
‡: Midfacial hypoplasia, cleft palate, hearing loss, articular abnormalities, cardiac abnormalities

Family	ID proband	Sex	Myopic grade*	Vitreous (age in yrs)	RRD eye	Age of onset RRD in yrs	Type of defect (age in yrs)	Other retinal abnormalities (age in yrs)	Extraocular abnormalities (age in yrs)‡
P94-161	I:1	M	-1/0	Normal (69)	OS	69	HS OS, RH OD (69)	Excavated optic disks, stretched and attenuated vessels, peripheral atrophy, pigmented lattice (69)	-
P01-2058	II:3	M	3	PVD of vitreous remnants, strongly attached to retina (38) after prior vitrectomy OD (34)	OD	13	? (13), MH (34), HS (38)	Lattice, sealed off with laser (34)	-
W00-428	II:4 †	F	2		ODS	22 (OD), 42 (OS)	? (22), HS (42)	Lattice (38)	-
W01-126	II:3	F	2/3	Threads with beads (31)	ODS	31	RH (31)	Retinoschizis, snailtracks, hyperpigmentations and chorioretinal atrophy (31)	-
W01-206	II:1	M	2	Normal (62)	OD	62	RH (62)	Chorioretinal atrophy, excavated and modest inversed papilla (62)	Hearing anamnestically reduced (65)
W02-140	II:1	F	0	Optically empty, threads without beads (77)	OD	63	RH (63)	Lattice, peripheral reticular hyperpigmentation (63)	Hearing anamnestically reduced (75)
W02-143	II:2	F	2	Degenerative (55), thin (72)	OD	55	RH (46 and 55)	Retinoschizis OS (34), starfold in retina OD (55), pseudomacular hole ODS (59 and 67)	CREST syndrome (37), perceptive high frequency hearing loss (>65)
W02-168	II:1	M	2	PVD, condensations (59)	OS	59	HS (59)	Swinging artery at defect causing small vitreous hemorrhage (59)	Moderate presbyacusis: symmetric perceptive hearing loss (61)
W03-032	III:3	F	3	Normal (21)	ODS	21	HS OD and RH ODS (21)	Lattice (18)	-
W03-036	II:3	M	0	Threads (59)	OS	59	HS ODS (59)	Lattice (59)	Presbyacusis ADS (63)
W03-053	II:2	M	3	Liquefied behind lens, PVD (80)	-	-	RH OD (77)	Peripapillar atrophy, peripheral retinal degeneration (56); macular RPE alterations OD (60); peripheral RPE alterations and lattice OS; bleak papilla, attenuated bloodvessels OD (80)	Hearing aid AD (76), AS (80)
W03-164	II:1	F	2		OS	24	RH (24)	High water marks in periphery OS, lattice and snailtrack in periphery OD (24)	-

Molecular genetic findings

COL2A1 exon 2 mutation analysis of the probands of the families with RRD included in this study failed to identify any nucleotide change in the coding region and in the flanking splice sites of this exon.
Linkage analysis leads to a maximum cumulative 2-point lod score of 0.28 (Zmax) at theta 0.2 for marker D12S85 (**Table 5.2**). Lod scores for each family included in this study are reported in **Table 5.3**. Cumulative 2-point lod scores calculated for a subgroup of families which showed positive lod scores values for all or some of the markers flanking the *COL2A1* gene (P94-161, P01-2058, W02-140, W03-032, and W03-053) did not reach statistical significance (Zmax = 2.31 at theta 0.000 for marker D12S339).

Table 5.2 Cumulative 2-point lod scores at the COL2A1 locus in 12 families with RRD

Marker	**Recombination Fraction**					
	0.000	0.100	0.200	0.300	0.400	0.500
D12S85	-2.93	0.10	0.28	0.21	0.09	0,00
D12S1701	-8.97	-0.95	-0.02	0.19	0.14	0,00
D12S1661	-7.84	-2.56	-1.15	-0.45	-0.11	0,00
D12S339	-7.15	-0.85	-0.05	0.25	0.17	0.00

Haplotype analysis failed to identify a single potential disease haplotype in the affected individuals of family W00-428, W01-126, W01-206, W02-143, W02-168, W03-036, and W03-164. In families W03-032 and W03-053, the putative disease haplotype was also present in two or more unaffected individuals. Finally, in family P01-2058, W02-140 and P94-161 haplotype analysis suggested linkage at the *COL2A1* locus (**Figure 5.2**). Sequence analysis of all 54 exons and flanking intron-exon boundaries of *COL2A1* in family P01-2058 and W02-140 did not reveal any pathological change in the coding region or at the exon splice sites of the *COL2A1* gene. In family P94-161 *COL2A1* mutation analysis is ongoing.

Table 5.3 Two-point lod scores at the COL2A1 locus in the 12 families with RRD included in this study

Family no.	Marker	Recombination Fraction					
		0.000	0.100	0.200	0.300	0.400	0.500
P94-161	D12S85	0,59	0,45	0,30	0,16	0,05	0,00
	D12S1701	0,59	0,45	0,30	0,16	0,05	0,00
	D12S1661	0,00	0,00	0,00	0,00	0,00	0,00
	D12S339	0,59	0,45	0,30	0,16	0,05	0,00
P01-2058	D12S85	0,42	0,31	0,20	0,10	0,03	0,00
	D12S1701	0,42	0,31	0,20	0,10	0,03	0,00
	D12S1661	0,00	0,00	0,00	0,00	0,00	0,00
	D12S339	0,42	0,31	0,20	0,10	0,03	0,00
W00-428	D12S85	0,05	0,04	0,03	0,02	0,01	0,00
	D12S1701	0,05	0,04	0,03	0,02	0,01	0,00
	COL2A1 c.504C>A	-1,37	-0,46	-0,21	-0,09	-0,02	0,00
	D12S1661	-1,36	-0,46	-0,21	-0,09	-0,02	0,00
	D12S339	-1,35	-0,42	-0,18	-0,06	-0,01	0,00
W01-126	D12S85	-1,76	-0,27	-0,04	0,03	0,04	0,00
	D12S1701	-1,77	-0,28	-0,05	0,03	0,04	0,00
	D12S1661	-1,46	-0,48	-0,22	-0,09	-0,02	0,00
	D12S339	0,37	0,31	0,25	0,17	0,09	0,00
W01-206	D12S85	-0,29	-0,22	-0,14	-0,07	-0,02	0,00
	D12S1701	-0,29	-0,20	-0,11	-0,04	-0,01	0,00
	D12S1661	-0,68	-0,21	-0,08	-0,03	-0,01	0,00
	D12S339	-0,29	-0,20	-0,11	-0,04	-0,01	0,00
W02-140	D12S85	0,01	0,01	0,00	-0,01	-0,01	0,00
	D12S1701	0,90	0,67	0,45	0,26	0,11	0,00
	D12S1661	-0,10	-0,06	-0,03	-0,01	0,00	0,00
	D12S339	1,21	0,93	0,64	0,36	0,14	0,00
W02-143	D12S85	-0,32	-0,20	-0,11	-0,05	-0,01	0,00
	D12S1701	-0,44	-0,25	-0,12	-0,05	-0,01	0,00
	D12S1661	-0,61	-0,36	-0,19	-0,08	-0,02	0,00
	D12S339	-0,60	-0,34	-0,18	-0,08	-0,02	0,00
W02-168	D12S85	0,00	0,00	0,00	0,00	0,00	0,00
	D12S1701	-1,80	-0,52	-0,22	-0,08	-0,02	0,00
	COL2A1 c.2195-55T>CG	-1,51	-0,42	-0,19	-0,07	-0,02	0,00
	D12S1661	-1,43	-0,31	-0,12	-0,04	-0,01	0,00
	D12S339	-1,83	-0,64	-0,30	-0,12	-0,03	0,00
W03-032	D12S85	0,05	0,00	-0,02	-0,01	0,00	0,00
	D12S1701	0,12	0,05	0,01	0,00	0,00	0,00
	COL2A1 c.2195-55T>CG	-0,34	-0,16	-0,07	-0,03	-0,01	0,00
	D12S1661	0,00	-0,01	-0,01	0,00	0,00	0,00
	D12S339	0,03	-0,02	-0,03	-0,02	-0,01	0,00
W03-036	D12S85	-2,35	-0,49	-0,23	-0,10	-0,03	0,00
	D12S1701	-4,94	-0,75	-0,30	-0,10	-0,02	0,00
	D12S339	-3,35	-0,44	-0,19	-0,08	-0,02	0,00
W03-053	D12S85	0,00	0,00	0,00	0,00	0,00	0,00
	D12S1701	0,07	0,04	0,02	-0,01	-0,01	0,00
	COL2A1 c.2195-55T>CG	0,43	0,32	0,21	0,11	0,03	0,00
	D12S1661	0,00	0,00	0,00	0,00	0,00	0,00
	D12S339	0,06	0,04	0,02	-0,01	-0,01	0,00
W03-164	D12S85	0,65	0,47	0,28	0,13	0,03	0,00
	D12S1701	-1,87	-0,54	-0,25	-0,10	-0,02	0,00
	D12S1661	-2,20	-0,68	-0,30	-0,12	-0,03	0,00
	D12S339	-2,41	-0,83	-0,38	-0,15	-0,04	0,00

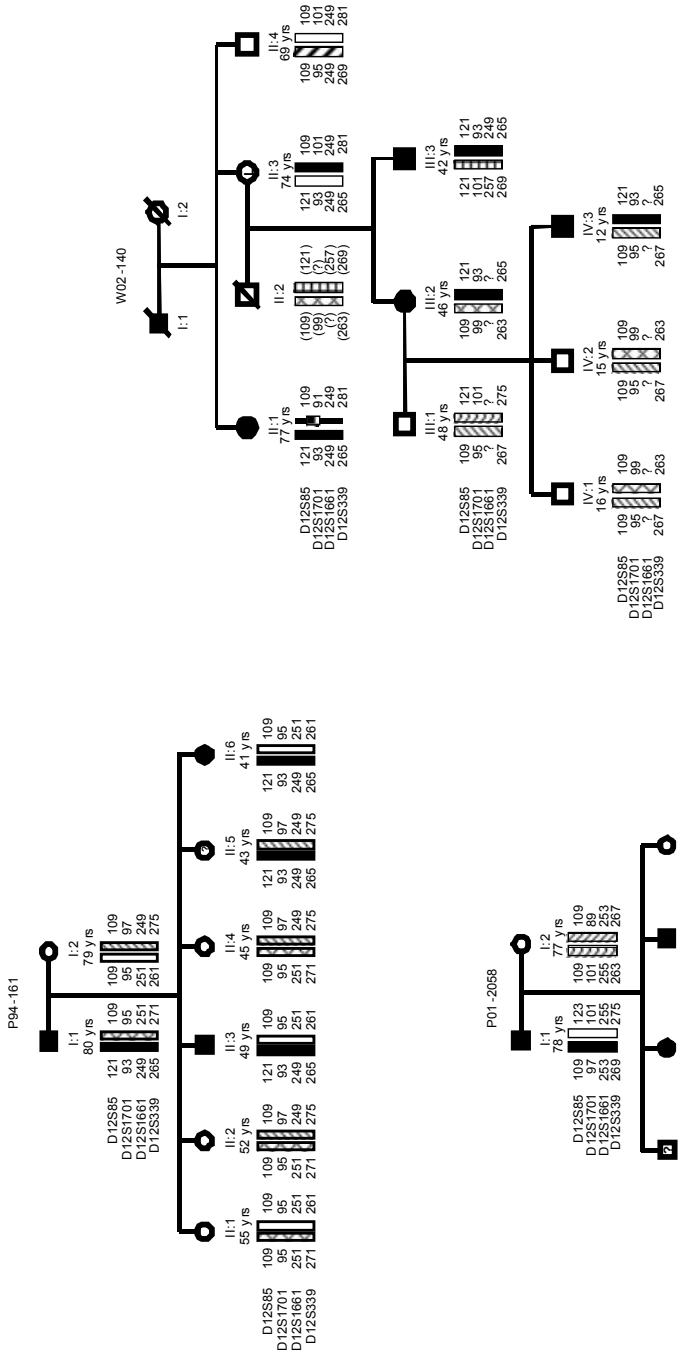

Figure 5.2 Linkage analysis in families P94-161, P01-2058 and W02-140

5.4 Discussion

RRD is a frequent ophthalmologic finding.[2-5] However, despite the relevance of RRD in clinical ophthalmology, an extensive analysis of nonsyndromic familial RRD has never been reported. In this study, we aimed to identify a sizeable group of RRD families, to assess the transmission of a mendelian trait, to describe the clinical features in familial RRD cases, and to investigate the role of the *COL2A1* gene in adRRD.
In general, the size of the recruited families was small, with 3-6 affected individuals each. Up to now, genetic analysis has only been reported for 12 large families with adRRD distinct from Wagner disease and other well defined vitreoretinopathies.[18;21-27] Nine of these families showed typical ocular abnormalities found in Stickler syndrome with absent or minimal systemic features, and were due to mutations generating a stop codon in exon 2 of the *COL2A1* gene.[21-26] The remaining three families also showed linkage at 12q13.11-q13.2, an area encompassing the *COL2A1* gene.[18;27] In one of these families (family B[18]) we found a stop codon mutation p.Arg453X in exon 30 of the *COL2A1* gene. In family A no pathologic change was detected in the coding sequence, in the flanking intron-exon boundaries, and in known regulatory regions present in intron 1 and at the 3'UTR of the *COL2A1* gene.[18] Finally a family with adRRD carrying a novel p.Gly118Arg mutation, also located outside the alternatively spliced exon 2 region of *COL2A1*, has been recently described by Richards et al.[27]
As all published adRRD families were associated with mutations in the *COL2A1* gene, we evaluated the role of *COL2A1* in our cohort of families. Sequence analysis failed to identify any pathologic variation in exon 2 of the *COL2A1* gene in any of the probands of the 12 RRD families described here. Statistical analysis of linkage data in the families presented in this study is not simple. The first difficulty is the small size of the families. Only in case of a major disease locus, significant cumulative lod scores can be expected. Moreover, in a number of cases the affection status could not be established, further limiting the informativity of some of the families. A second drawback, is the frequent occurrence of late-onset RRD, which limits the informativity of the younger generations. We have taken this into account in the calculation of the lod scores by estimating the penetrance as a function of the age of onset. Penetrance at 75 years of age was assumed to be 95% based on data of families with RRD and *COL2A1* mutations[18;26 and refs therein] and family A in Go et al.[18], which all showed a high or complete penetrance above 70 years of age. Moreover, to account for the possible occurrence of "non-genetic or non-mendelian" cases of RRD in our families, an age-dependent function for phenocopies was introduced in our model. Cumulative 2-point lod scores excluded *COL2A1* as the disease locus in the majority of the 12 families with RRD. Moreover, lod scores did not reach statistical significance for any subgroup of these families. However, due to the small size of the families, lod score data could not exclude the involvement of *COL2A1* in some of the families. In particular, haplotype analysis suggested linkage with *COL2A1* for family P01-2058, W02-140 and P94-

161. For this reason, probands of these families underwent sequence analysis of the entire *COL2A1* gene. However, the failure to identify a pathological change in the protein coding sequence and the sequences flanking the exons of the *COL2A1* gene in at least two of these families, strongly suggests that *COL2A1* is not involved in the RRD pathology in these families.
The identification of other monogenic causes of RRD through whole genome genetic linkage analysis is only feasible if only one or very few major loci exist.

5.5 Acknowledgments

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References

1. Ivanisevic M. The natural history of untreated rhegmatogenous retinal detachment. *Ophthalmologica* 1997;211:90-2.
2. Schepens CL, Marden D. Data on the Natural History of Retinal Detachment: I. Age and Sex Relationships. *Arch.Ophthalmol.* 1961;66:631-42.
3. Wilkes SR, Beard CM, Kurland LT, Robertson DM, O'Fallon WM. The incidence of retinal detachment in Rochester, Minnesota, 1970-1978. *Am.J.Ophthalmol.* 1982;94:670-3.
4. Tornquist R, Stenkula S, Tornquist P. Retinal Detachment - A Study of A Population-Based Patient Material in Sweden 1971-1981. 1. Epidemiology. *Acta Ophthalmol.(Copenh).* 1987;65:213-22.
5. Go SL, Hoyng CB, Klaver CCW. Genetic risk of rhegmatogenous retinal detachment: a familial aggregation study. *Arch.Ophthalmol.* 2005;123:1237-1241.
6. Rowe JA, Erie JC, Baratz KH, Hodge DO, Gray DT, Butterfield L et al. Retinal detachment in Olmsted County, Minnesota, 1976 through 1995. *Ophthalmology* 1999;106:154-9.
7. Risk factors for idiopathic rhegmatogenous retinal detachment. The Eye Disease Case-Control Study Group. *Am.J.Epidemiol.* 1993;137:749-57.
8. Byer NE. Natural history of posterior vitreous detachment with early management as the premier line of defense against retinal detachment. *Ophthalmology* 1994;101:1503-13.
9. Murakami-Nagasako F, Ohba N. Phakic retinal detachment associated with atrophic hole of lattice degeneration of the retina. *Graefes Arch.Clin.Exp.Ophthalmol.* 1983;220:175-8.
10. Lewis H. Peripheral retinal degenerations and the risk of retinal detachment. *Am.J.Ophthalmol.* 2003;136:155-60.
11. Lois N, Wong D. Pseudophakic retinal detachment. *Surv.Ophthalmol.* 2003;48:467-87.
12. Snead MP, Yates JR. Clinical and Molecular genetics of Stickler syndrome. *J.Med. Genet.* 1999;36:353-9.
13. Parentin F, Sangalli A, Mottes M, Perissutti P. Stickler syndrome and vitreoretinal degeneration: correlation between locus mutation and vitreous phenotype. Apropos of a case. *Graefes Arch.Clin.Exp.Ophthalmol.* 2001;239:316-9.
14. Wilkin DJ, Mortier GR, Johnson CL, Jones MC, De Paepe A, Shohat M et al. Correlation of linkage data with phenotype in eight families with Stickler syndrome. *Am.J.Med.Genet.* 1998;80:121-7.
15. McLeod D, Black GC, Bishop PN. Vitreous phenotype: genotype correlation in Stickler syndrome. *Graefes Arch.Clin.Exp.Ophthalmol.* 2002;240:63-5.

16. Richards AJ, Baguley DM, Yates JR, Lane C, Nicol M, Harper PS et al. Variation in the vitreous phenotype of Stickler syndrome can be caused by different amino acid substitutions in the X position of the type II collagen Gly-X-Y triple helix. *Am.J.Hum.Genet.* 2000;67:1083-94.

17. Martin S, Richards AJ, Yates JR, Scott JD, Pope M, Snead MP. Stickler syndrome: further mutations in COL11A1 and evidence for additional locus heterogeneity. *Eur. J.Hum.Genet.* 1999;7:807-14.

18. Go SL, Maugeri A, Mulder JJS, van Driel MA, Cremers FPM, Hoyng CB. Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the COL2A1 gene. *Invest.Ophthalmol.Vis.Sci.* 2003;44:4035-43.

19. Brunner HG, van Beersum SE, Warman ML, Olsen BR, Ropers HH, Mariman EC. A Stickler syndrome gene is linked to chromosome 6 near the COL11A2 gene. *Hum. Mol.Genet.* 1994;3:1561-4.

20. Sirko-Osadsa DA, Murray MA, Scott JA, Lavery MA, Warman ML, Robin NH. Stickler syndrome without eye involvement is caused by mutations in COL11A2, the gene encoding the alpha2(XI) chain of type XI collagen. *J.Pediatr.* 1998;132:368-71.

21. Richards AJ, Martin S, Yates JR, Scott JD, Baguley DM, Pope FM et al. COL2A1 exon 2 mutations: relevance to the Stickler and Wagner syndromes. *Br.J.Ophthalmol.* 2000;84:364-71.

22. van der Hout AH, Verlind E, Scheffer H, Hooymans JMM. A new family with a mutation in exon 2 of COL2A1 and Stickler syndrome without systemic manifestations. *Am.J.Hum.Genet.* 2001;69:623.

23. Gupta SK, Leonard BC, Damji KF, Bulman DE. A frame shift mutation in a tissue-specific alternatively spliced exon of collagen 2A1 in Wagner's vitreoretinal degeneration. *Am.J.Ophthalmol.* 2002;133:203-10.

24. Donoso LA, Edwards AO, Frost AT, Ritter R, Ahmad NN, Vrabec T et al. Identification of a stop codon mutation in exon 2 of the collagen 2A1 gene in a large Stickler syndrome family. *Am.J.Ophthalmol.* 2002;134:720-7.

25. Parma ES, Korkko J, Hagler WS, Ala-Kokko L. Radial perivascular retinal degeneration: a key to the clinical diagnosis of an ocular variant of Stickler syndrome with minimal or no systemic manifestations. *Am.J.Ophthalmol.* 2002;134:728-34.

26. Donoso LA, Edwards AO, Frost AT, Ritter R, Ahmad N, Vrabec T et al. Clinical variability of Stickler syndrome: Role of exon 2 of the collagen COL2A1 gene. *Surv. Ophthalmol.* 2003;48:191-203.

27. Richards AJ, Meredith S, Poulson A, Bearcroft P, Crossland G, Baguley DM et al. A novel mutation of COL2A1 resulting in dominantly inherited rhegmatogenous retinal detachment. *Invest.Ophthalmol.Vis.Sci.* 2005;46:663-8.

28. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16:1215.

Chapter

6

General discussion

6.1 Mutations in the *COL2A1* gene as a cause for RRD

As shown in **Chapters 4** and **5**, we have studied two large families (families A and B) and twelve smaller multiplex families with nonsyndromic RRD. In both families A and B, we found statistically significant linkage with a region on chromosome 12 containing the *COL2A1* gene. This is depicted in **Figure 6.1**.

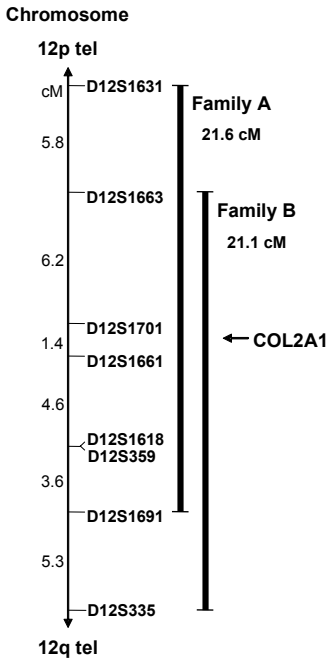

Figure 6.1 Linkage intervals of the RRD in families A and B
Genetic distances in centiMorgans (cM)

In humans, at least 27 collagen types are described, each of which consists of three polypeptide chains (α-chains), and each chain containing at least one domain of a repeating Gly-X-Y sequence. They can be subdivided in 9 subgroups, mainly on the basis of their structure and supramolecular organization. One of these subgroups consists of fibril-forming collagens and, among others, contains the type II and type XI collagens.[1;2] Collagens of this subgroup can aggregate into highly orientated suprastructures of staggered arrangements of individual collagen monomers.[3]
Type II collagen is found in cartilaginous and ocular tissue (vitreous and cornea). The collagen type XI is found in collagen type II containing tissues, and forms the core of

collagen type II.[4;5] Due to their torsional stability and tensile strength, these collagen types lead to integrity, and yet flexibility, of the tissues they are found in.[1]
The *COL2A1* gene codes for a specific α-chain, called the α1(II)-chain. In collagen type II, three identical α1(II)-chains together form the typical trimer. Type XI collagen, unlike type II collagen, consists of three different α-chains. One of these chains consists of an unprocessed form of the α1(II)-chain encoded by *COL2A1*, which differs only in the extent of glycosylation and hydroxylation.[1] The different α-chain compositions of collagen type II and collagen type XI are shown in **Figure 6.2**.

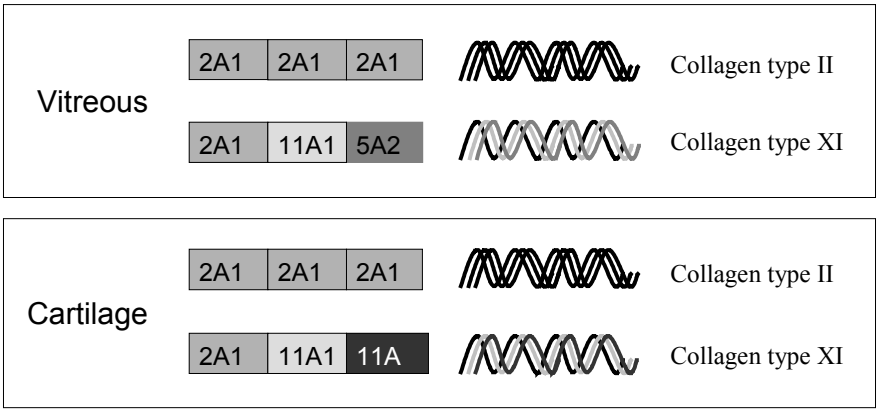

Figure 6.2 Schematic α-chain compositions of collagen type II and collagen type XI in the vitreous body compared to cartilage

Sequence analysis of the *COL2A1* gene was performed in both families. In family B, an Arg453Ter mutation in the *COL2A1* gene was found. This mutation leads to nonsense-mediated decay of the mRNA and thus in COL2A1 haploinsufficiency in patients. Similar premature terminations causing haploinsufficiency in Stickler patients have been described.[6] The exact Arg453Ter mutation had previously been reported in a sporadic patient with the type II Stickler syndrome, showing all classic abnormalities of the syndrome. Despite known variability of expression in Stickler syndrome, in a family as large as family B, the haploinsufficiency was predicted to result in systemic abnormalities in several patients, which was not the case.
A predominantly ocular subtype of the Stickler syndrome with mutations in *COL2A1* had been reported in several families.[7-11] **Table 6.1** lists patient characteristics of 10 families with no or less extra-ocular manifestations with mutations in the *COL2A1* gene. One family also showing minimal extra-ocular abnormalities with a mutation in exon 2 of the *COL2A1* gene was presented as a Wagner disease family, but the clinical description of the patients lacks crucial information to completely exclude a

predominantly ocular Stickler syndrome.[12]
The reason for the predominantly ocular expression of the Stickler syndrome was sought in alternative splicing of exon 2 in ocular tissue in comparison to cartilaginous tissue. In the latter, exon 2 is thought to be spliced out during the maturation process of the protein, resulting in collagen type IIB (see **Figure 6.3**), while in ocular tissue it is thought to remain part of the protein (type IIA). In cartilage, both type IIA and IIB are found, but the collagen type IIB predominates, whereas in the eye type IIA predominates. Therefore, a mutation in exon 2 is hypothesized to mainly affect the eye.[7;13;14] However, the mutation found in family B without systemic abnormalities of Stickler syndrome was found outside exon 2, residing in exon 30.

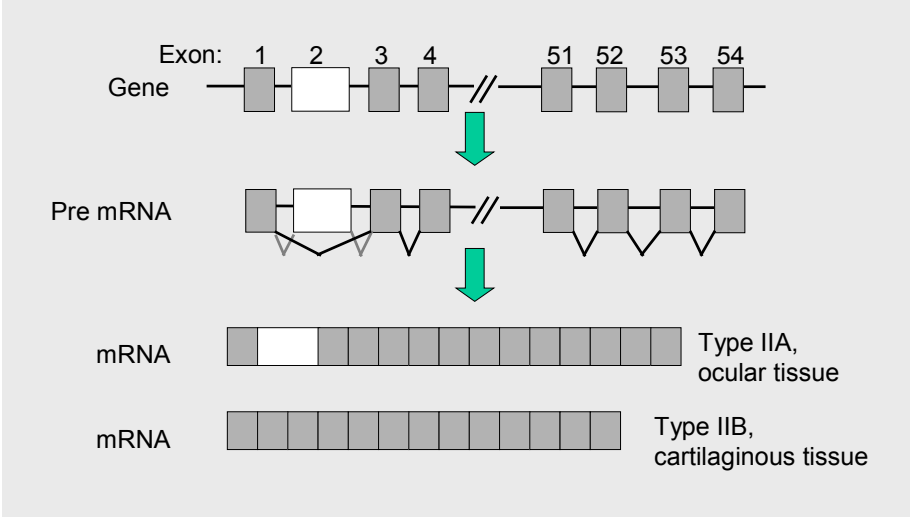

Figure 6.3 Alternative splicing of exon 2 of the *COL2A1* gene

Table 6.1 Phenotypic characteristics of families with RRD with minimal or no systemic features with mutations in the COL2A1 gene, presented in occurrences per number of described patients.

Family/reference	Effect in COL2A1 propeptide	Myopia			RD	Age of onset first RRD (yrs)	Orofacial abnormalities			Hearing loss	Articular symptoms	
		Mild	Moderate	Severe			Midfacial hypoplasia	Cleft or high-arched palate			Hypermobility	Abnormalities
COL2A1 propeptide (containing exon 2)												
MS11/Richards[7]	I54fsX59	-	-	4/5	4/5	ND	-	-		2/5	2/5	-
MS13/Richards[7]	V56fsX57	1/5	-	3/5	3/5	ND	1/5	1/5		2/5	-	-
MS62/Richards[7]	T47X	-	2/6	1/6	5/6	ND	5/6	2/6		3/6	-	1/6
Dutch family/Van der Hout[8]	C64X	ND	ND	ND	ND	ND	-	-		-	-	ND
Donoso[9;11]	C86X	ND	ND	ND	95/165	5-46, mean 15.2	-	1/95		2/28	ND	2/28
Southeastern US family*/ Parma[10]	C86X	7/41	=combined	34/41	43/66	4-48, mean of 70%: [11]	-	3/100		1/40	4/32	1/38
"Wagner disease"/Gupta[12]	C57X	-	-	9/9	50/55 (including retinal tears)	ND	-	-		ND	ND	ND, no spondyl-arthrosis
COL2A1 mature protein												
"Wagner disease"/ Körkkö[15]	G267D	-	2/4	-	4/4	8-28, mean of 13	3/4	-		-	2/4	1/4
Family B/Go[16]	R653X	1/13	5/13	6/13	8/13	6-27, mean 16.9	2/13	-		-	-	-
MS68/Richards[17]	G318R	ND	ND	ND	ND, but high incidence	ND	-	-		-	-	-

Note that the Dutch families of Van der Hout and Go reveal very few systemic abnormalities
ND = not described; *This family is very probably related to the family of Donoso and shares surnames and geographical area

The afore-mentioned suggests that at least one other favourable genetic factor must be involved in family B. We postulate two possibilities for the localisation of this factor. 1. Family B belongs to a relatively closed religious community in a specific region in the Netherlands. Therefore, a possible shared genetic background within this community could have become more pronounced in this family. The favourable genetic factor(s) could then reside anywhere in the genome. 2. The genetic factor resides near the *COL2A1* gene. It is known that the regulation of expression of most genes occurs at the level of transcription, but it can also occur downstream in the complex posttranslational modification of COL2A1. Transcription factors generally bind within 200 base pairs at the 5' end of a gene. They are generally thought to be *trans*-acting, and act on both gene copies, as opposed to the regulatory promoter elements, in which *cis*-acting regulation on the nearby located gene is expected.[18] However, the greater the distance between the genetic co-factor and the *COL2A1* gene, the greater the chance that meiotic crossovers will disrupt the link between *COL2A1* and the genetic co-factor. In family B, such a disruption could result in the full-blown Stickler syndrome in future offspring. Hypothetically, the genetic co-factor could lead to an upregulation of the transcription of the protein from the normal (wildtype) *COL2A1* allele, partially compensating for the haploinsufficiency. Alternatively, there could be a downregulation of the expression of genes that, together with the COL2A1 protein, form the trimeric collagen type XI, the *COL11A1* and *COL11A2* genes. These two possibilities are depicted in **Figure 6.4** (*see next page*).

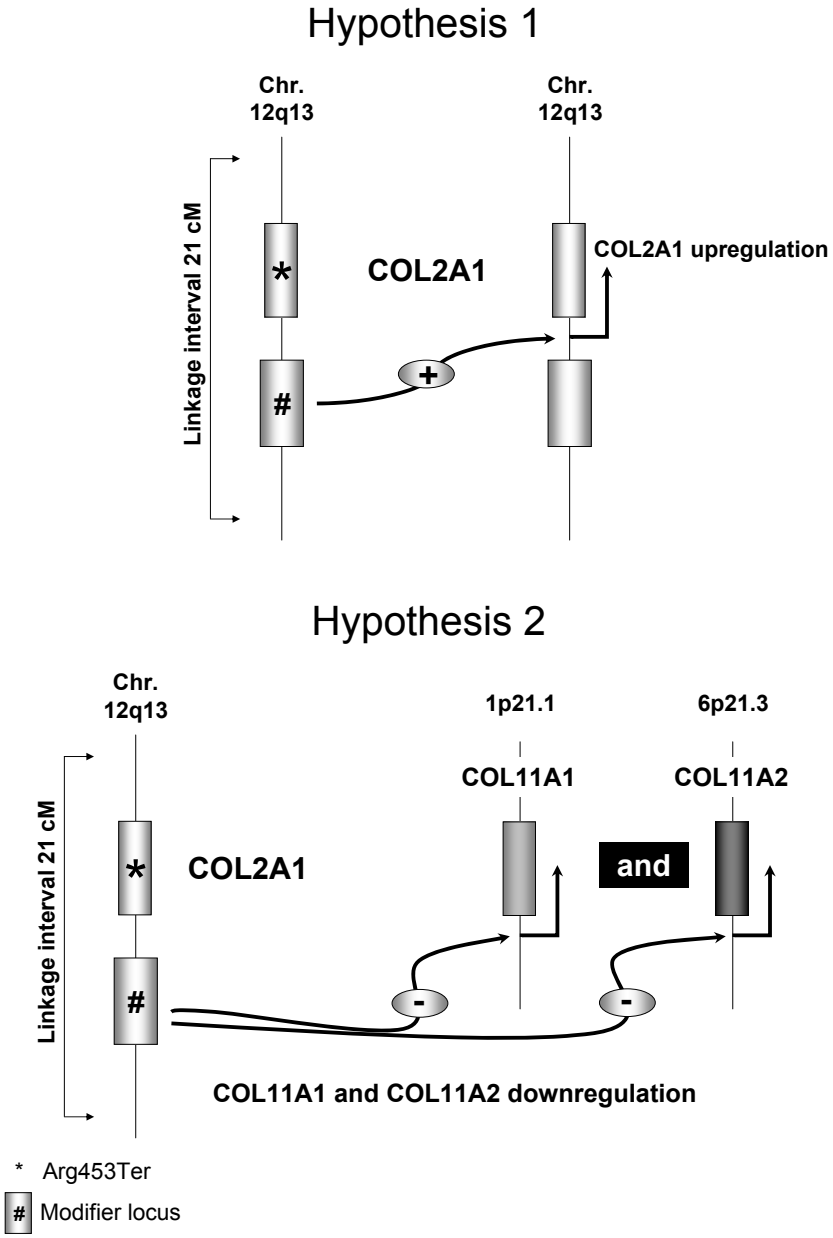

Figure 6.4 The effect of modifier factors on the transcription of collagen genes in family B

A known gene in the vicinity of *COL2A1*, encoding a transcription factor with a regulatory effect on the *COL2A1* gene, is the *SP1* gene. *SP1* induces the transcription of the α1(II) procollagen of the *COL2A1* gene. *Sp1* knock-out mice show retarded development and lethality around 11 days post-coitum, and *SP1* overexpression studies have demonstrated partial repair of the chondrocyte phenotype.[19;20] It is possible that this gene is overexpressed in family B, resulting in a close to normal chondrocytic repair, leaving patients of this family with a close to normal phenotype except for an abnormal vitreous. Alternatively, abnormalities in other transcription factors with a downregulating ability on the binding of SP1, like for example SP3 (Sp3 transcription factor) and IL1B (interleukin 1, beta), could also be involved.[20;21]

This theory would, in a similar fashion, also explain the inter- and intrafamilial variability of Stickler syndrome. The question is whether this hypothetical genetic factor co-segregating with the *COL2A1* mutation in family B has any relationship with the cause of RRD in family A.

In family A, we have sequenced all exons of the *COL2A1* gene with at least 30 base pairs of the flanking intron sequence. We failed to find the causative mutation. Possibly, we have missed a mutation residing far away in one of the incompletely sequenced introns, a mutation in the promoter region, or a mutation outside the gene. A mutation in the afore-mentioned putative co-factor resulting in an incomplete function could also have caused the retinal detachments.

Recently, a second report of an autosomal dominant RRD family without systemic abnormalities of Stickler syndrome with a mutation outside exon 2 was published. The identified Gly118Arg mutation in this family resided in exon 15. Missplicing due to the mutation was excluded as an explanation for the mild phenotype, and it was concluded that the mutation seems to disrupt the collagen helix and/or fibril formation less than expected from other missense mutations that result in severely systemically affected phenotypes due to a dominant negative effect.[17]

6.2 The role of the *COL2A1* gene in smaller multiplex RRD families

Based on linkage analysis, in three of the twelve smaller families, the genetic defect could reside in or near the *COL2A1* gene. In the nine remaining smaller multiplex families, the involvement of *COL2A1* has not been demonstrated. This implies that more than one gene is involved in RRD. Unlike the situation in other tissues, in the vitreous body the *COL11A2* derived third α-chain of collagen type XI is replaced by a *COL5A2* derived α-chain. The *COL5A2* gene therefore is a good candidate gene for linkage analysis in RRD families.
As hypothesized for family B, other similar pathways involving co-factors could possibly be involved in other subtypes of the Stickler syndrome with ocular abnormalities. For this reason, the possible involvement of *COL11A1* and *COL5A2* in the development of RRD in these families will also be investigated, in addition to the in **Chapter 4** described candidate genes or loci.
However, there is another explanation for exclusion of *COL2A1* (and other genes). The smaller families consist of only 2 to 7 affected individuals. Due to this limited number of affected individuals, there is a chance that in some of these families the cause for RRD is polygenic, which stresses the importance of epidemiological and statistical data, as described in the next paragraph.

6.3 Epidemiology and clinical implications

In **Chapter 2**, it was shown that a genetic predisposition for nonsyndromic RRD exists, apart from myopia. This genetic component of RRD is mainly expressed in a limited number of families who show multiple first degree relatives with RRD. Two out of 181 families (1.2%) with an affected proband show two or more affected relatives (siblings and children), whereas only one out of 408 control families (0.2%) has at least two relatives with a retinal detachment. (See **Table 2.3**) The affected relatives demonstrate an onset at younger ages than the relatives of controls, but a statistically significant difference was reached only for ages of 69 years or higher.
A comparison with other ophthalmic disorders in which genetic factors have been investigated is difficult, but seems interesting to outline the magnitude of the genetic risk identified. Comparison with age-related macular degeneration seems inappropriate because the phenotype, unlike the retinal detachment as known so far, is influenced by environmental factors, such as diet and smoking.[22] Therefore, the parameters of comparison would be unclear.
A comparison with primary open-angle glaucoma would be more appropriate, although also in this disorder the causative mechanism has not completely been identified. A positive family history for glaucoma is considered as a major risk factor in the development of primary open angle glaucoma, because the prevalence of glaucoma in siblings (10.4%) and offspring (1.1%) of glaucoma patients is considerably higher than in those of controls (0.7% and 0.0% respectively).[23] The prevalence rate for siblings and offspring of nonsyndromic RRD patients (13/1090= 1.2%) was also higher than the prevalence rate for those of unaffected controls (9/2345= 0.4%). The estimated lifetime absolute risk at 80 years of age is 9.2 times higher for relatives of glaucoma patients compared to relatives of healthy controls, whereas in nonsyndromic RRD the lifetime absolute risk at 85 years of age is only 2.6 times higher.[23] These data suggest that RRD has a smaller genetic component than primary open-angle glaucoma.
The magnitude of the genetic predisposition by RRD proband status is also less than the genetic predisposition due to myopia, as shown in **Chapter 2** and as compared with a more recent family aggregation study in high myopia estimates a sibling recurrence risk of 10.0%, whereas the sibling recurrence risk ratio was estimated 4.9%, based on the age of onset of spectacle wear, which was assumed to correlate with the final degree of myopia. Unfortunately, in that study offspring was not studied.[24] A negative refractive error appears to enhance the risk of RRD among relatives both in the case group and the control group, but the risk is highest for relatives of RRD patients. This may indicate that myopia genes can interact with other genetic determinants, or in this case, with the RRD genes. The actual myopia genes are yet unknown, but in a recent study using a genomewide scan in dizygotic twins loci were found on chromosomes 3q26, 4q12, 8p23 and 11p13 (amongst others harbouring the *PAX6* gene).[25] For high myopia, five different loci are currently known: chromosome Xq28, 7q36,

12q21-q23, 17q21-q22 and 18p11.31.[26-30] For the more common mild to moderate myopia, another recent study has shown linkage evidence on chromosome 22q12.[31] Interestingly, interaction between myopia and proband status in our study was not statistically significant, implying that these two factors exist mainly independent of each other, and by deduction, that myopia and RRD may involve different genes. This conclusion was supported by reports of families with autosomal dominant RRD with a large range of refractive errors from slightly hyperopic to severe myopia, or even without myopia.[16;32;33] Based on our data, however, it cannot be excluded that some RRD genes will be found within the same loci as those of myopia, or will even involve the same genes.

6.4 Clinical implications and future studies

Studies investigating the natural course of rhegmatogenous retinal detachments have revealed that they often lead to blindness if the condition remains untreated.[34] From the two large families studied in **Chapter 3** we have concluded that autosomal dominantly inherited RRD appears at very young ages in comparison to the age of common, mostly sporadic RRD that appears mostly around the age of 50-60 years.[35-37]

Another important difference with the regular RRD is the predominant presentation with multiple atrophic holes, whereas in the common RRD the predominant retinal break is the horseshoe-shaped tear. The atrophic hole, on the other hand, is generally regarded as relatively harmless and does not very often require treatment.[38]

Furthermore, the patients in our study have a relatively bad prognosis, needing multiple surgical procedures to achieve retinal reattachment. In the majority of cases, visual acuities do not exceed 20/63 in the end. Common RRD prognoses are relatively better in the sense that they need less reoperations: 76-85% of the RRD patients has an attached retina after one surgical procedure, whereas in families A and B a mean number of 2.4 and 2.5 reoperations was necessary. The final visual acuity in common, sporadic RRD is 6/18 or better in 46%.[39] In macula-off RRD the mean final visual acuity is 6/24, and a visual acuity of 6/12 or better is seen in 31-47%.[40]

With the mutation identified in the *COL2A1* gene in one of these families, the possibility of genetic screening and counselling in this family has become available. Another benefit from our study lies in possible prevention of RRD in carriers of the *COL2A1* mutation, for example with lasercoagulation or even preventive surgery. A recent study has demonstrated that preventive lasercoagulation of the entire 360 degrees of the peripheral retina reduces the risk of bilaterality of RRD with 12.2% in fellow eyes when compared to untreated fellow eyes of RRD patients.[41]

In family B, genetic counselling has already been applied in some relatives, and in some young patients with the *COL2A1* gene defect lasercoagulation has even been applied to prevent the children from the very likely development of RRD and its surgical consequences.

A prospective study will be necessary to reveal the best method of prevention in inherited RRD. The necessary preventive approach may differ from the common, sporadic RRD, because it appears that in hereditary RRD a considerable part of the problem may reside in the affected, atrophic peripheral retina causing the round atrophic holes, which may add to the risk of myopia and degeneration of the vitreous body.

Preliminary data from a histopathological study we performed, showed COL2A1 protein staining in the wall of only peripheral retinal vessels, as shown in **Figure 6.5**. The result was reproduceable in multiple slides and retinal specimens, but needs further investigation.

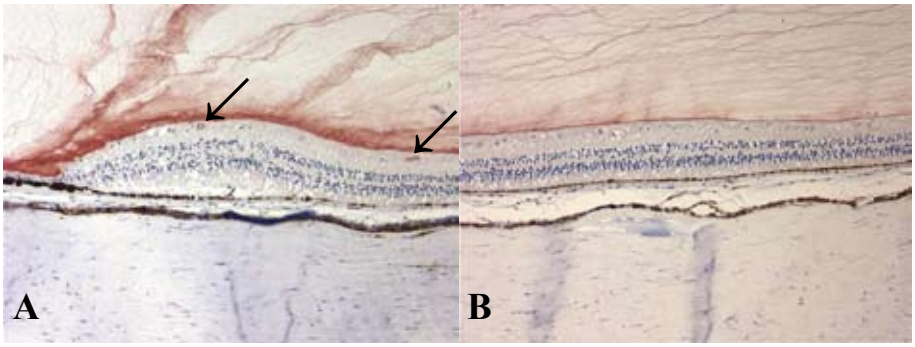

Figure 6.5 COL2A1 protein staining in the superficial retinal bloodvessels
Note the staining in the peripheral superficial bloodvessel walls (arrows) (**A**), as opposed to the absence of staining of the more centrally located vessels in the retina (**B**).

However, the role of the COL2A1 protein in these vessels is still unclear, but possibly the lack of COL2A1 in the peripheral retinal vessels leads to a lesser quality of the construction or the maintenance of the peripheral retinal tissue, leading to peripheral atrophic holes. The cause of the known peripheral radial perivascular pigmentation in Stickler patients could possibly also be related to abnormalities of these vessels. Further histological studies of retinal tissue of healthy individuals and Stickler patients are required to explore this theory. Another approach to explore the magnitude of the theory could involve experiments of expression of the *COL2A1* gene in tissue samples of the retina from different embryological stages, from healthy individuals and from Stickler syndrome patients.

References

1. Gelse K, Poschl E, Aigner T. Collagens - structure, function, and biosynthesis. *Adv. Drug.Deliv.Rev.* 2003;55:1531-46.
2. Myllyharju J, Kivirikko KI. Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet.* 2004;20:33-43.
3. Hulmes DJ. The collagen superfamily--diverse structures and assemblies. *Essays Biochem.* 1992;27:49-67.
4. Brown JC, Timpl R. The collagen superfamily. *Int.Arch.Allergy Immunol.* 1995;107:484-90.
5. Myllyharju J, Kivirikko KI. Collagens and collagen-related diseases. *Ann.Med.* 2001;33:7-21.
6. Wilkin DJ, Liberfarb R, Davis J, Levy HP, Cole WG, Francomano CA et al. Rapid determination of COL2A1 mutations in individuals with Stickler syndrome: analysis of potential premature termination codons. *Am.J.Med.Genet.* 2000;94:141-8.
7. Richards AJ, Martin S, Yates JR, Scott JD, Baguley DM, Pope FM et al. COL2A1 exon 2 mutations: relevance to the Stickler and Wagner syndromes. *Br.J.Ophthalmol.* 2000;84:364-71.
8. van der Hout AH, Verlind E, Scheffer H, Hooymans JMM. A new family with a mutation in exon 2 of COL2A1 and Stickler syndrome without systemic manifestations. *Am.J.Hum.Genet.* 2001;69:623.
9. Donoso LA, Edwards AO, Frost AT, Ritter R, Ahmad NN, Vrabec T et al. Identification of a stop codon mutation in exon 2 of the collagen 2A1 gene in a large Stickler syndrome family. *Am.J.Ophthalmol.* 2002;134:720-7.
10. Parma ES, Korkko J, Hagler WS, Ala-Kokko L. Radial perivascular retinal degeneration: a key to the clinical diagnosis of an ocular variant of Stickler syndrome with minimal or no systemic manifestations. *Am.J.Ophthalmol.* 2002;134:728-34.
11. Donoso LA, Edwards AO, Frost AT, Ritter R, Ahmad N, Vrabec T et al. Clinical variability of Stickler syndrome: Role of exon 2 of the collagen COL2A1 gene. *Surv. Ophthalmol.* 2003;48:191-203.
12. Gupta SK, Leonard BC, Damji KF, Bulman DE. A frame shift mutation in a tissue-specific alternatively spliced exon of collagen 2A1 in Wagner's vitreoretinal degeneration. *Am.J.Ophthalmol.* 2002;133:203-10.
13. Ryan MC, Sandell LJ. Differential expression of a cysteine-rich domain in the amino-terminal propeptide of type II (cartilage) procollagen by alternative splicing of mRNA. *J.Biol.Chem.* 1990;265:10334-9.
14. Sandell LJ, Morris N, Robbins JR, Goldring MB. Alternatively Spliced Type II Procollagen mRNAs Define Distinct Populations of Cells during Vertebral Development: Differential Expression of the Amino-Propeptide. *J.Cell Biol.* 1991;114:1307-19.

15. Korkko J, Ritvaniemi P, Haataja L, Kaariainen H, Kivirikko KI, Prockop DJ et al. Mutation in type II procollagen (COL2A1) that substitutes aspartate for glycine alpha 1-67 and that causes cataracts and retinal detachment: evidence for molecular heterogeneity in the Wagner syndrome and the Stickler syndrome (arthro-ophthalmopathy). *Am.J.Hum. Genet.* 1993;53:55-61.

16. Go SL, Maugeri A, Mulder JJS, van Driel MA, Cremers FPM, Hoyng CB. Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the COL2A1 gene. *Invest.Ophthalmol.Vis.Sci.* 2003;44:4035-43.

17. Richards AJ, Meredith S, Poulson A, Bearcroft P, Crossland G, Baguley DM et al. A novel mutation of COL2A1 resulting in dominantly inherited rhegmatogenous retinal detachment. *Invest.Ophthalmol.Vis.Sci.* 2005;46:663-8.

18. Mueller RF, Young ID. *Emery's elements of medical genetics*. 11th ed. Churchill Livingstone, 2001: 1-372.

19. Marin M, Karis A, Visser P, Grosveld F, Philipsen S. Transcription factor Sp1 is essential for early embryonic development but dispensable for cell growth and differentiation. *Cell* 1997;89:619-28.

20. Ghayor C, Chadjichristos C, Herrouin JF, Ala-Kokko L, Suske G, Pujol JP et al. Sp3 represses the Sp1-mediated transactivation of the human COL2A1 gene in primary and de-differentiated chondrocytes. *J.Biol.Chem.* 2001;276:36881-95.

21. Tan L, Peng H, Osaki M, Choy BK, Auron PE, Sandell LJ et al. Egr-1 mediates transcriptional repression of COL2A1 promoter activity by interleukin-1beta. *J.Biol. Chem.* 2003;278:17688-700.

22. Klaver, C. C. W. Genetic epidemiologic studies on age-related maculopathy. A population-based approach. 1-150. 2000.

23. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch.Ophthalmol.* 1998;116:1640-5.

24. Farbrother JE, Kirov G, Owen MJ, Guggenheim JA. Family aggregation of high myopia: Estimation of the sibling recurrence risk ratio. *Invest.Ophthalmol.Vis.Sci.* 2004;45:2873-8.

25. Hammond CJ, Andrew T, Mak YT, Spector TD. A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genomewide scan of dizygotic twins. *Am.J.Hum.Genet.* 2004;75:294-304.

26. Schwartz M, Haim M, Skarsholm D. X-linked myopia: Bornholm eye disease. Linkage to DNA markers on the distal part of Xq. *Clin.Genet.* 1990;38:281-6.

27. Naiglin L, Gazagne C, Dallongeville F, Thalamas C, Idder A, Rascol O et al. A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. *J.Med.Genet.* 2002;39:118-24.

28. Young TL, Ronan SM, Alvear AB, Wildenberg SC, Oetting WS, Atwood LD et al. A second locus for familial high myopia maps to chromosome 12q. *Am.J.Hum.Genet.* 1998;63:1419-24.

29. Paluru P, Ronan SM, Heon E, Devoto M, Wildenberg SC, Scavello G et al. New locus for autosomal dominant high myopia maps to the long arm of chromosome 17. *Invest. Ophthalmol.Vis.Sci.* 2003;44:1830-6.

30. Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS et al. Evidence that a locus for familial high myopia maps to chromosome 18p. *Am.J.Hum. Genet.* 1998;63:109-19.

31. Stambolian D, Ibay G, Reider L, Dana D, Moy C, Schlifka M et al. Genomewide linkage scan for myopia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 22q12. *Am.J.Hum.Genet.* 2004;75:448-59.

32. Haut J, Limon S, Offret H, Morax S. [Familial peripheral retinal degenerescence and retinal detachment]. *Bull.Soc.Ophtalmol.Fr.* 1973;73:27-37.

33. Saari KM. Autosomal dominant juvenile vitreoretinal degeneration and retinal detachment. *Int.Ophthalmol.* 1986;9:45-60.

34. Ivanisevic M. The natural history of untreated rhegmatogenous retinal detachment. *Ophthalmologica* 1997;211:90-2.

35. Rowe JA, Erie JC, Baratz KH, Hodge DO, Gray DT, Butterfield L et al. Retinal detachment in Olmsted County, Minnesota, 1976 through 1995. *Ophthalmology* 1999;106:154-9.

36. Schepens CL, Marden D. Data on the natural history of retinal detachment. Further characterization of certain unilateral nontraumatic cases. *Am.J.Ophthalmol.* 1966;61:213-26.

37. Scott JD. Prevention and Perspective in Retinal-Detachment. *Eye* 1989;3:491-515.

38. American Academy of Ophthalmology. Preferred Practice Pattern: Posterior Vitreous Detachment, Retinal Breaks, and Lattice Degeneration. Available from http://www.aao.org/aao/education/library/ppp. 2003.

39. Ah-Fat FG, Sharma MC, Majid MA, McGalliard JN, Wong D. Trends in vitreoretinal surgery at a tertiary referral centre: 1987 to 1996. *Br.J.Ophthalmol.* 1999;83:396-8.

40. Ross WH, Kozy DW. Visual recovery in macula-off rhegmatogenous retinal detachments. *Ophthalmology* 1998;105:2149-53.

41. Avitabile T, Bonfiglio V, Reibaldi M, Torrisi B, Reibaldi A. Prophylactic treatment of the fellow eye of patients with retinal detachment: a retrospective study. *Graefes Arch. Clin.Exp.Ophthalmol.* 2004;242:191-6.

Chapter 7

Summaries

7.1 Summary

Rhegmatogenous retinal detachment (RRD) is the most common type of retinal detachment. In this type of retinal detachment, a break in the neurosensory part of the retina allows fluid from the vitreous cavity to enter the space between the sensory retina and the retinal pigment epithelium. Yearly, approximately 1 in 10,000 individuals presents with this disorder, and most RRDs occur between the ages of 40 and 70 years. The fellow eye of affected individuals has a 4.2-19% risk of RRD development in the future. Whereas asymptomatic operculated and atrophic holes are generally considered quite harmless, flap tears and the less common retinal dialyses often progress to RRDs. Risk factors like myopia, vitreous degeneration, lattice degeneration, trauma and lens surgery can contribute to the development of RRD.
Untreated, RRD leads to blindness in the majority of cases. However, laser treatment and cryocoagulation in cases of retinal breaks without subretinal fluid and/or surgery in more advanced cases can often prevent further detachment or achieve reattachment of the retina. An anatomically good result can be achieved in about 95% of all cases.
In several nonsyndromic (e.g. myopia and Wagner disease) and syndromic inherited ophthalmic disorders (e.g. Stickler syndrome), retinal detachment is one of the disease features. Due to overlapping clinical features and the fact that many of the causative gene mutations are still unknown, it is sometimes difficult to distinguish between some of these disorders.

The aim of this thesis was to elucidate the genetic basis of retinal detachment, and subsequently to contribute to a better understanding of the disease and counselling of retinal detachment patients and their families. This may enable prevention or early treatment of the disorder in selected cases. We used different approaches: first, in an epidemiological study, we investigated the magnitude of the genetic risk of nonsyndromic RRD and the risk of relatives of RRD patients independent of other risk factors. Secondly, a clinical approach was used to investigate possible differences in presentation or in surgical prognosis in two large families with autosomal dominant nonsyndromic retinal detachments (families A and B). Finally, a genetic approach was used to investigate the genes involved in retinal detachment families, first in the two large autosomal dominant nonsyndromic retinal detachment families, and subsequently in 12 smaller families in order to investigate the extent of involvement of the *COL2A1* gene in these.

To estimate the risk of RRD in relatives of RRD patients, we studied 2345 relatives of 408 healthy individuals (‘controls’) from a Dutch Caucasian population and 1090 relatives of 181 patients with RRD (‘cases’), as described in **Chapter 2**. The yearly incidence of RRD in the Netherlands is 8.24 per 100,000 individuals. Thirteen relatives (1.2%) with RRDs were found in 10 case probands, while 9 relatives (0.4%)

of 8 control probands were diagnosed with RRD. The cumulative lifetime risk of RRD was 7.7% for the case relatives and 3.0% for control relatives, yielding a 2.6 times higher risk for RRD in case relatives. Myopia did not fully explain this increased risk. We concluded that familial occurrence of RRD is a risk factor for RRD, especially in families with more than two affected first degree relatives in two generations.

In surgery for retinal detachment, primary reattachment of the retina is achieved in 76-85% of the cases, but as described earlier, an overall anatomically satisfactory result is eventually achieved in 90-95%. Surgery in inherited disorders like Stickler syndrome and Wagner disease, however, is frequently disappointing and recurrences of retinal detachments due to proliferative vitreoretinopathy occur frequently.
To investigate the surgical results in nonsyndromic inherited RRD, we studied the affected members of families A and B with an autosomal dominantly inherited RRD without classic ocular or systemic features of RRD-related syndromes, as described in **Chapter 3**. Medical records with an average of 7 years follow-up were retrospectively reviewed with a focus on preoperative, operative and postoperative data.
The average age of onset in both families was lower than the age of onset in sporadic RRD cases, but was comparable with the age of onset of retinal detachment in heritable disorders. RRD in fellow eyes was found more often than expected from studies in sporadic RRD. The detachments predominantly resulted from a high number of round, atrophic holes, rather than the horseshoe tears as characteristically encountered in sporadic RRD. Again this was more comparable to the defects in heritable disorders with RRD like high myopia, Stickler syndrome and Kniest dysplasia. These results are suggestive of an intrinsic retinal fragility.
Furthermore, poor surgical results were found, independent of the surgical techniques used. In about half of the cases, mostly due to proliferative vitreoretinopathy formation, multiple operations were needed to reattach the retina. In some patients the retina remained detached. Final visual acuities were on average 20/80 in family A and 20/50 in family B, with a visual acuity of hand motions or worse in three out of ten patients of each family. The outcome of surgery pointed out the necessity of timely recognition of individuals at risk in autosomal dominant RRD families, and the need for prophylactic treatment of even asymptomatic round atrophic retinal defects in these cases. Because of the apparent intrinsic fragility of the peripheral retina, the reported posterior vitreous detachment after laser treatment and the high prevalence of proliferative vitreoretinopathy in our patients, we suggest that gentle prophylactic laser treatment should be applied to the peripheral retina, covering all quadrants in patients at risk.

In **Chapter 4**, we reported the result of the linkage analysis we performed in families A and B, in order to find the defective gene(s) involved. The analysis was performed in 28 individuals (15 affected) from family A and 22 individuals (12 affected) from family B. We first analyzed the three known loci for high myopia, the Wagner disease/

erosive vitreoretinopathy locus, the nonsyndromic congenital retinal nonattachment locus and the predominantly ocular Stickler syndrome gene *COL2A1*. Significant LOD scores for both families were found in a region containing the *COL2A1* gene on chromosome 12. In family A, no mutations in the coding regions and exon splice-sites of *COL2A1* were found. In family B, however, an Arg453Ter mutation was found in this gene. The finding of this mutation was in two ways surprising: firstly, family B showed no Stickler characteristics, but this exact mutation and other similar protein truncating mutations until then had been associated with the classic Stickler syndrome. Secondly, reported families with a predominantly ocular phenotype of the syndrome, had always been associated with protein truncating mutations in exon 2, whereas this mutation was found in exon 30.

In **Chapter 5** we investigated the role of the *COL2A1* gene in the development of RRD in 12 smaller families. *COL2A1* exon 2 and flanking splice-sites sequence analysis in all probands did not reveal any mutations. Linkage studies with markers from the region encompassing the *COL2A1* gene showed cosegregation of the chromosomal region harbouring the *COL2A1* gene in three families (families P01-2058, W02-140 and P94-161). However, sequence analysis of the *COL2A1* gene coding region and the intron-exon boundaries in at least two of the three families (P01-2058 and W02-140) did not reveal any mutation. These data suggest that the *COL2A1* gene is not the major affected gene in smaller RRD families. In families P01-2058, W02-140 and family A, a mutation in a gene in the vicinity of *COL2A1* cannot be excluded. In family A, the pathologic DNA sequence variant can be localized in any of the genes residing in the 21.6 cM region encompassing the *COL2A1* gene. Furthermore, the exclusion of linkage with the *COL2A1* region in at least 9 families indicates that at least one other locus outside this region must exist. Alternatively, RRD in these families is caused by several, and possibly even non-genetic, factors.

7.2 Samenvatting

Het meest voorkomende type netvliesloslating is de rhegmatogene netvliesloslating (RRD). Bij dit type netvliesloslatingen komt er via een defect in het neurosensorische deel van de retina vocht uit de glasvochtruimte tussen de sensorische retina en het retinaal pigmentepitheel terecht. Ongeveer 1 op de 10.000 mensen per jaar krijgen de aandoening. De meeste RRD's treden op bij een leeftijd van tussen de 40 en 70 jaar. Het tweede oog van patiënten heeft een risico van 4.2-19% op een toekomstige netvliesloslating. Terwijl asymptomatische atrofische gaten en gaten met operculum (klepje) als vrij onschuldig worden gezien, vindt er bij flapscheuren en bij de in mindere mate voorkomende orascheuren vaker voortschrijding tot RRD plaats. Risicofactoren als myopie, glasvochtdegeneratie, lattice degeneratie, trauma en lenschirurgie kunnen bijdragen aan de vorming van RRD.
Als de RRD onbehandeld blijft, eindigt deze in de meerderheid van de gevallen in blindheid. Laserbehandeling en cryocoagulatie in gevallen met retinale defecten zonder subretinaal vocht en/of chirurgie in de meer voortgeschreden gevallen kan verdere loslating voorkomen, of het netvlies weer aanliggend doen maken. Een anatomisch goed resultaat wordt in ongeveer 95% van alle gevallen behaald.
In een aantal niet-syndromale (zoals bv. myopie en de ziekte van Wagner) en syndromale erfelijke oogaandoeningen (zoals bv. het Stickler syndroom) is een netvliesloslating een van de kenmerken. Doordat de aandoeningen klinische overlap vertonen en doordat veel van de ziekte veroorzakende genmutaties nog niet gevonden zijn, is het soms moeilijk de aandoeningen onderling van elkaar te onderscheiden.

Het doel van dit proefschrift was om de genetische basis van netvliesloslatingen te belichten, om daardoor bij te kunnen dragen aan een beter begrip van de aandoening en patiënten met netvliesloslatingen en hun families beter te kunnen voorlichten. Hierdoor kan er in bepaalde gevallen in de toekomst mogelijk preventie of behandeling in een vroeg stadium plaatsvinden. Er werden verschillende benaderingswijzen gebruikt: allereerst een epidemiologische studie, waarin we de omvang van het genetische risico van niet-syndromale RRD en het risico voor familieleden van RRD patiënten onafhankelijk van andere risicofactoren onderzochten. Vervolgens werd een klinische benadering aangewend om mogelijke verschillen in presentatie of in chirurgische prognose te onderzoeken in twee grote families met autosomaal dominant overervende niet-syndromale netvliesloslatingen (families A en B). Ten slotte volgde een genetische benadering, waarbij onderzocht werd welke genen betrokken waren bij familiaire netvliesloslatingen. Dit gebeurde eerst in de twee grote families A en B, en vervolgens, om te achterhalen in hoeverre het *COL2A1*-gen in deze betrokken was, in 12 kleinere families.

Om het risico op RRD in familieleden van RRD patiënten te kunnen inschatten,

bestudeerden we 2345 familieleden van 408 gezonde individuen (“controles”) uit een Nederlandse Caucasische populatie en 1090 familieleden van 181 patiënten met RRD (“cases”) (**Hoofdstuk 2**). De jaarlijkse incidentie van RRD in Nederland bedraagt 8,24 op de 100.000 personen. Dertien familieleden (1,2%) van 10 case probands bleken RRD’s te hebben, terwijl slechts 9 familieleden (0,4%) van 8 control probands een RRD hadden. Het cumulatieve risico voor RRD was 7,7% voor de familieleden van cases en 3,0% voor familieleden van controles, hetgeen resulteerde in een 2,6 keer verhoogd risico op RRD voor familieleden van cases. Dit verhoogde risico kon niet geheel door myopie verklaard worden. We concludeerden dat het voorkomen van RRD in de familie een separate risicofactor is voor RRD, met name in families met meer dan twee aangedane eerstegraads familieleden in twee generaties.

Bij chirurgie voor netvliesloslatingen resulteert een eenmalige behandeling in 76-85% in een aanliggend netvlies, maar na meerdere operaties wordt uiteindelijk in 90-95% een anatomisch bevredigend resultaat bereikt. Bij erfelijke aandoeningen zoals het Stickler syndroom en de ziekte van Wagner zijn de chirurgische resultaten echter vaak teleurstellend en komen er door proliferatieve vitreoretinopathie vaak recidieven van de netvliesloslatingen voor.
Om de chirurgische resultaten bij niet-syndromaal overervende RRD te onderzoeken, bestudeerden we de aangedane familieleden uit families A en B met autosomaal dominant overervende RRD zonder de klassieke kenmerken van RRD-gerelateerde syndromen (**Hoofdstuk 3**). We onderzochten retrospectief de medische gegevens, met een gemiddelde follow-up tijd van 7 jaar. Hierbij werd speciale aandacht besteed aan pre-operatieve, operatieve en post-operatieve data.
De gemiddelde beginleeftijd van de RRD’s in beide families was lager dan de gemiddelde beginleeftijd van sporadische RRD patiënten, maar hij was vergelijkbaar met de beginleeftijd van netvliesloslatingen in erfelijke aandoeningen. RRD in het tweede oog werd vaker aangetroffen dan op grond van studies in sporadische RRD verwacht werd. De RRD’s onstonden met name door een groot aantal ronde, atrofische gaten in plaats van door de flapscheuren die gewoonlijk worden aangetroffen bij sporadische RRD. Ook dit gegeven was meer vergelijkbaar met de defecten in erfelijke aandoeningen met RRD, zoals hoge myopie, het Stickler syndroom en Kniest dysplasie. Deze resultaten suggereerden een intrinsieke fragiliteit van het netvlies.
Verder werden er, onafhankelijk van de gebruikte chirurgische technieken, tegenvallende resultaten gevonden. In ongeveer de helft van het aantal gevallen waren er meerdere operaties nodig om het netvlies aanliggend te maken, meestal doordat er proliferatieve vitreoretinopathie optrad. Bij sommige patiënten bleef het netvlies afliggend. De uiteindelijke gezichtsscherpte was gemiddeld 0,25 in familie A en 0,4 in familie B, waarbij in drie van de tien patiënten van elke familie slechts waarneming van handbewegingen of minder restte. De chirurgische resultaten toonden het belang van tijdige herkenning van individuen met een verhoogd risico in families met autosomaal dominant overervende RRD aan, maar ook dat zelfs bij asymptomatische,

ronde, atrofische retinadefecten profylactische behandeling nodig is in deze gevallen. Vanwege de klaarblijkelijke intrinsieke fragiliteit van de perifere retina, de bekende achterste glasvochtmembraanloslating na lasertherapie en het frequente voorkomen van proliferatieve vitreoretinopathie in onze patiënten, stellen wij voor bij patiënten met een verhoogd risico alle kwadranten van de perifere retina voorzichtig met lasercoagulatie te behandelen.

In **Hoofdstuk 4** werd het resultaat van het koppelingsonderzoek dat wij in families A en B verrichtten om de betrokken gendefecten te achterhalen weergegeven. De analyse werd verricht in 28 individuen (15 aangedanen) van familie A en 22 individuen (12 aangedanen) van familie B. We onderzochten eerst de drie reeds bekende loci voor hoge myopie, het locus voor de ziekte van Wagner/erosieve vitreoretinopathie, het locus voor niet-syndromale, congenitale retinale 'nonattachment' en het bij 'predominantly ocular' Stickler syndroom betrokken *COL2A1*-gen. In beide families werden significante LOD scores gevonden in een regio rond het *COL2A1*-gen op chromosoom 12. In familie A werden geen mutaties in de coderende gebieden en de exon splice-sites van *COL2A1* gevonden. In familie B werd echter wel een Arg453Ter mutatie in dit gen gevonden. Deze bevinding was verrassend: ten eerste omdat familie B geen Stickler kenmerken vertoonde hoewel deze exacte mutatie en vergelijkbare mutaties die resulteren in onvolledig gesynthetiseerde eiwitten tot die tijd geassocieerd werden met het klassieke Stickler syndroom. Ten tweede werden families met een 'predominantly ocular' fenotype van het Stickler syndroom tot dusverre altijd met mutaties in exon 2 geassocieerd, waarbij eveneens de COL2A1-translatie prematuur stopt, terwijl de gevonden mutatie in exon 30 aangetroffen werd.
In **Hoofdstuk 5** onderzochten we de rol van het *COL2A1*-gen in de ontwikkeling van RRD in 12 kleinere families. Sequentie-analyse van exon 2 van het *COL2A1*-gen en de flankerende splice-sites liet geen mutaties zien. Koppelingsstudies met markers van de regio rond het *COL2A1*-gen lieten cosegregatie van de chromosomale regio met daarin het *COL2A1*-gen zien in drie families (families P01-2058, W02-140 en P94-161). Sequentie-analyse van de coderende gebieden van het *COL2A1*-gen en de intron-exon overgangen in ten minste twee van de drie families (P01-2058 en W02-140) toonde echter geen mutaties aan. Deze data geven aan dat het *COL2A1*-gen niet het belangrijkste aangedane gen is in kleinere RRD families. In families P01-2058, W02-140 en familie A kan een mutatie in een gen in de buurt van *COL2A1* niet worden uitgesloten. In familie A zal de pathologische DNA-sequentievariant zich in een van de andere genen in de 21.6 cM regio rond het *COL2A1*-gen bevinden. Verder suggereert het ontbreken van koppeling met de *COL2A1* regio in tenminste 9 families dat er minstens één ander locus buiten deze regio moet bestaan. Het is echter ook mogelijk dat de RRD in deze families wordt veroorzaakt door meerdere, mogelijk zelfs niet-genetische, factoren.

Chapter 8

Appendices

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Curriculum vitae

Sioe Lie Go werd op 7 november 1974 te Boxmeer geboren. Haar lagere school doorliep zij in vijf jaren in Afferden (Limburg), waarna zij op 11-jarige leeftijd aanving met haar middelbare school op het Elzendaalcollege te Boxmeer. Haar gymnasium-bèta opleiding voltooide zij daar in 1992. Vervolgens studeerde zij Geneeskunde aan de Katholieke Universiteit Nijmegen, alwaar zij in 1999 afstudeerde.
Tijdens haar studie was zij actief betrokken bij wetenschappelijke onderzoeksprojecten op de afdelingen Interne geneeskunde, Orthopedie en Oogheelkunde van het Academisch Ziekenhuis Nijmegen (later het Universitair Medisch Centrum St Radboud genaamd). Daarnaast volgde zij van 1993 tot 1996 de opleiding Acupunctuur en Chinese geneeskunde aan de Nederlandse Artsen Acupunctuur Vereniging, verrichtte zij correctiewerk voor het tijdschrift Acupunctuur van de Nederlandse Artsen Acupunctuur Vereniging, was zij actief als EHBO-instructrice en later EHBO-commissielid aan de Medische Faculteitsvereniging Nijmegen, en als penningmeester en later voorzitter van de Werkgroep Integratie Geneeswijzen te Nijmegen.
Na een AGNIO-schap op de afdeling Oogheelkunde van eerdergenoemd ziekenhuis in 1999 ving zij aan met het onderzoeksproject naar de erfelijkheid van netvliesloslatingen, dat in deze dissertatie resulteerde. Sioe Lie is inmiddels bezig met haar specialisatie tot oogarts. Zij is sinds 1998 getrouwd met Milan Tjioe.

Publications

Articles:

Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the *COL2A1* gene (S.L. Go, A. Maugeri, J.J.S. Mulder, M.A. van Driel, F.P.M. Cremers, C.B. Hoyng, *Invest.Ophthalmol.Vis.Sci. 2003;44:4035-4043*)

Three families displaying the combination of Stargardt disease with cone-rod dystrophy and retinitis pigmentosa (B.J. Klevering, A. Maugeri, A. Wagner, S.L. Go, C. Vink, F.P.M. Cremers, C.B. Hoyng, *Ophthalmology 2004;111:546-553*)

Autosomal Dominant Rhegmatogenous Retinal Detachment – Clinical Appearance and Surgical Outcome (T. Theelen, S.L. Go, M.A.D. Tilanus, B.J. Klevering, A.F. Deutman, F.P.M. Cremers, C.B. Hoyng, *Graefes Arch.Clin.Exp.Ophthalmol. 2004;242:892-897*)

Genetic risk of rhegmatogenous retinal detachment: a familial aggregation study (S.L. Go, C.B. Hoyng, C.C.W. Klaver, *Arch.Ophthalmol. 2005;123:1237-1241*)

Abstracts:

Koppelingsonderzoek bij een familie met autosomaal dominant overervende ablatio retinae [Abstract] (S.L. Go, M.A. van Driel, A.F. Deutman, H.G. Brunner, F.P.M. Cremers, C.B. Hoyng, *Programma 195e jaarvergadering NOG 2001*, pag. 92. Abstract 63A)

Genetische risico van ablatio retinae [Abstract] (J. van Haarlem, S.L. Go, F.P.M. Cremers, C.B. Hoyng, *Programma 195e jaarvergadering NOG 2001*, pag. 98. Abstract 69A)

Linkage analysis in a family with autosomal dominant retinal detachment [ARVO abstract] (S.L. Go, M.A. van Driel, A.F. Deutman, H.G. Brunner, F.P.M. Cremers, C.B. Hoyng, *Invest Ophthalmol Vis Sci*. 2001;42:S648. Abstract 3483)

Phenotype and Surgical Outcome in Autosomal Dominant Rhegmatogenous Retinal Detachments [Abstract] (S.L. Go, T. Theelen, M.A.D. Tilanus, B.J. Klevering, A.F. Deutman, F.P.M. Cremers, C.B. Hoyng, 2003 A*nnual Meeting Abstract and Program Planner [on CD-ROM], Association for Research in Vision and Ophthalmology,*

Abstract 2989)

Het klinisch beloop van ablatio retinae in twee autosomaal dominante niet-syndromale ablatio retinae families [Abstract] (S.L. Go, T. Theelen, A.F. Deutman, C.B. Hoyng, *Programma 196e jaarvergadering NOG 2002*, pag. 68. Abstract 43A)

Candidate Genes Analysis in Autosomal Dominant Rhegmatogenous Retinal Detachment [Abstract] (A.Mukhopadhyay, A.Maugeri, S.L. Go, C.B. Hoyng, F.P.M. Cremers, 2005 A*nnual Meeting Abstract and Program Planner [on CD-ROM], Association for Research in Vision and Ophthalmology,* Abstract 5487)

Posters:

Linkage analysis in a family with autosomal dominant retinal detachment [poster] (S.L. Go, M.A. van Driel, A.F. Deutman, H.G. Brunner, F.P.M. Cremers, C.B. Hoyng, *presented at the Association for Research in Vision and Ophthalmology 2001*)

Drie families waarin de ziekte van Stargardt gecombineerd voorkomt met kegel-staaf dystrofie of retinitis pigmentosa [poster] (B.J. Klevering, A. Maugeri, A. Wagner, S.L. Go, C. Vink, F.P.M. Cremers en C.B. Hoyng, *Programma 196e jaarvergadering NOG 2002*, pag. 117, Poster EE)

Phenotype and Surgical Outcome in Autosomal Dominant Rhegmatogenous Retinal Detachments [poster] (S.L. Go, T. Theelen, M.A.D. Tilanus, B.J. Klevering, A.F. Deutman, F.P.M. Cremers, C.B. Hoyng, *presented at the Association for Research in Vision and Ophthalmology 2003*)

Candidate Genes Analysis in Autosomal Dominant Rhegmatogenous Retinal Detachment [poster] (A.Mukhopadhyay, A.Maugeri, S.L. Go, C.B. Hoyng, F.P.M. Cremers, *presented at the Association for Research in Vision and Ophthalmology 2005*)

Colour plates

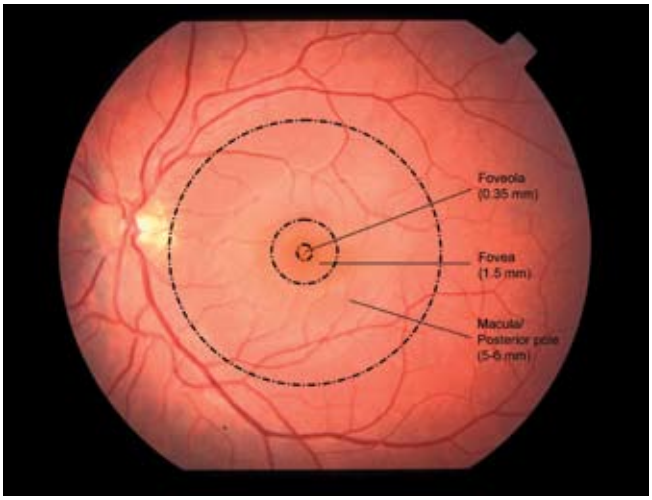

Figure 1.6 The positions of macula, fovea and foveola in the posterior pole of the eye

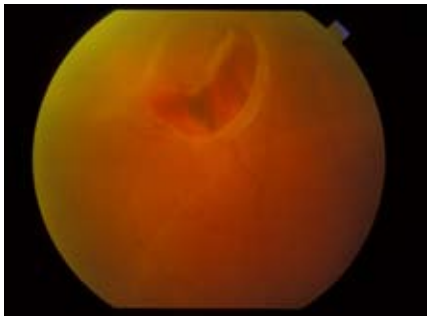

Figure 1.8 Retinal detachment caused by a flap tear

Figure 1.12 Complete retinal detachment.
Note that the papilla centers all folds.

Figure 1.14 Preretinal circular line at the equator in a patient with Wagner disease (arrow)

Figure 1.15 Situs inversus of the central retinal vessels in a patient with Wagner disease

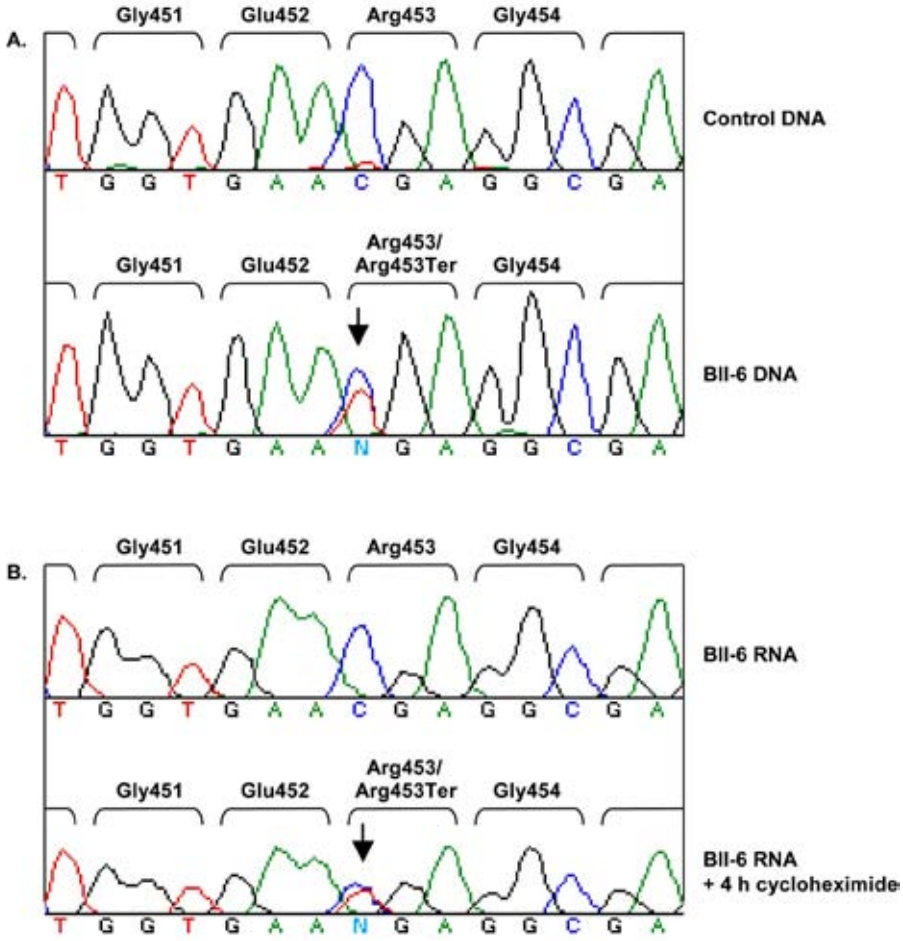

Figure 4.2 *COL2A1* mutation analysis in patient BII-6
(**A**) DNA sequence analysis of part of exon 30 (previously denoted as exon 28) of the *COL2A1* gene. Top: sequence of a control individual; bottom: sequence of the clinically affected individual BII-6, carrying a heterozygous C-to-T transition resulting in an Arg453Ter mutation. (**B**) RNA analysis of the Arg453Ter mutation in patient BII-6. Top: sequence of the cDNA of the patient. Because of mRNA instability, the transcript of the mutant allele was not detectable. Bottom: sequence of the cDNA of the patient obtained after incubation of lymphoblastoid cells in a medium containing cycloheximide, which prevents nonsense-mediated mRNA decay. Sequence analysis shows presence of transcripts from both the normal and the mutant allele.

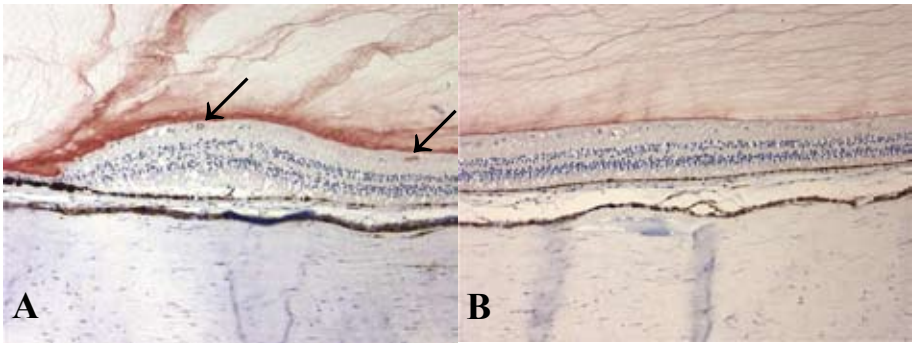

Figure 6.5 COL2A1 protein staining (red) in the superficial retinal bloodvessels
Note the staining in the peripheral superficial bloodvessel walls (arrows) (**A**), as opposed to the absence of staining of the more centrally located vessels in the retina (**B**).